

DEVELOPMENT OF FEEDING PROTOCOLS FOR
TILAPIA RENDALLI IN MALAWI REARED IN
SEMI-INTENSIVE CULTURE SYSTEMS

CENTRE FOR NEWFOUNDLAND STUDIES

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A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN
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**DEVELOPMENT OF FEEDING PROTOCOLS FOR *TILAPIA RENDALLI* IN
MALAWI REARED IN SEMI-INTENSIVE CULTURE SYSTEMS**

By

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**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN
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ABSTRACT

Tilapia is the major group of fish used in aquaculture in Malawi and its potential has never been realised. Previous research has focussed on improving the growth performance. Nutritional studies were carried out to determine and improve feeding protocols in order to enhance the growth of *Tilapia rendalli*. The first experiment was on the effect of using different organic manure. *T. rendalli* in the chicken manure treatment were significantly larger and had higher net annual yields than those in cattle, pig manure and no-manure treatments. Significantly higher amounts of chlorophyll *a* and numbers of zooplankton were found in ponds fertilized with chicken manure.

In the second experiment, *T. rendalli* were evaluated in ponds fertilized with chicken manure to determine the effect of using different single ingredient supplements. *T. rendalli* in the soybean treatment were significantly larger and had higher net yields than in maize bran, rice bran and unsupplemental, chicken manure treatments.

The third experiment was set up to determine the effect of different temperatures. *T. rendalli* in the 32° C treatment were significantly larger than those in 24, 28° C and ambient temperatures. Fish cultured at 32° C had significantly higher digestibility coefficients, lower feed conversion ratios, higher feed conversion efficiency and protein efficiency ratios. The last experiment examined the effect of different salinities. *T. rendalli* in the 10‰ grew significantly larger than those in the freshwater, 5‰, and 15‰. Fish cultured in 10‰ salinity had significantly higher digestibility coefficients, lower feed conversion ratios, higher feed conversion efficiency and protein efficiency ratios.

The survival of the fish was significantly different among salinity levels decreasing from 100% in freshwater to 66.7 % in 15‰ salinity treatment.

The overall results suggest that the use of chicken manure would produce better results than cattle and pig manure treatments, and ponds fertilized with chicken manure may be supplemented with soybean to increase yield. Water temperature of 32° C and salinity level of 10‰ may be optimal for *T. rendalli* because feed conversion was higher, and digestibility coefficients were higher for protein, fat, ash and gross energy under these conditions.

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Chapter 1

INTRODUCTION AND OVERVIEW

1.1 Introduction

Malawi is a landlocked country in the southern part of Africa. It is situated between 9 and 17° S bordered by Zambia in the west, Mozambique in the southeast and Tanzania in the north. Malawi's total area is 118,484 km² and about 20% of this is covered by water (Government of Malawi, GOM- Department of Environmental Affairs, 1998). Malawi's fishery is based on capture fisheries estimated at about 60,000 metric tonnes per year. The fisheries production from the wild is declining and with an increase in human population the pressure is even greater on the resource. Fish provide about 70% of the animal protein consumed, but fish consumption has declined from 14.7 kg/capita/year in 1970 to less than 7.0 kg/capita/year in the 1990s (GOM-Fisheries Department, 1998).

To supplement the declining production of fish from capture fisheries, the Malawi Government has embarked on developing aquaculture at both smallholder and commercial levels (GOM-Fisheries Department, 1997). The aquaculture potential of Malawi is one of the highest in the Southern African Development Community (SADC) region (Dickson and Brooks, 1997). Malawi's aquaculture is just developing and the need to develop this sector has been documented in government policy as an alternative source of protein that will ease the pressure on the wild resource (GOM-Fisheries Department, 1999).

Aquaculture represents one of the world's fastest growing food producing sectors providing a product that is an acceptable supplement to wild fish and plants (FAO, 2000). Malawi's level of production is very low which may be attributed to several factors

including: lack of suitable species adapted to the environment; lack of infrastructure characterised by "traditional" fish farming; no established marketing arrangements; and no regular supply of high quality feed.

1.2 Overview and background information

Malawi needs appropriate feeding strategies in rural or subsistence aquaculture farming activities to meet part of the growing animal protein needs of the rural population (Tacon, 1991). Little research has been done in fish nutrition in the aquaculture sector to assure its sustainability at the smallholder level. Some work has been done on the utilisation of organic and inorganic fertilizers with some supplementation (Kadongola, 1991; Brummett and Noble, 1995; Jamu and Costa-Pierce, 1995). It is, however, important to continue to explore the development of feeding strategies that utilize locally available agricultural and industrial by-products to improve fish production in aquaculture as feed, either in the form of fertilizers, pond food organisms or dry diet. This is an essential component for the success of fish farming activity (Tacon, 1991).

1.2.1 Aquaculture in Malawi

Compared with many African countries, a considerable volume of work has been conducted on aquaculture in Malawi. These include research, infrastructural development, extension and manpower training for both aquaculture and for fisheries in general (ICLARM-GTZ, 1991). The development of aquaculture in Malawi is growing in part due to new approaches to project design and implementation that began in Southern

Africa in the late 1980s by the Food and Agriculture Organization (FAO) of the United Nations, the German Agency for Technical Cooperation (GTZ) and the International Center for Living Aquatic Resources Management (ICLARM) (Brummett and Costa-Pierce, 2002). Lately, the Japanese International Cooperation Agency (JICA) has been developing some indigenous species for small-scale aquaculture in Malawi (Ohashi *et al.*, 1999) and the Canadian International Development Agency (CIDA) has assisted the Southern African Development Community (SADC) in aquaculture development. Aquaculture research and training activities are part of the regional aquaculture development project implemented at the University of Malawi, where Bunda College of Agriculture and Marine Institute (MI) of the Memorial University of Newfoundland are partners (RADP, 1999).

Despite these efforts, the production of fish from aquaculture has been static at 331.7 Mt/year from 1992 to 2001 (FAO, 2003) (Appendix 1.1). This production come from smallholder farmers mainly through village based aquaculture (Brummett and Costa-Pierce, 2002). These effects have been hampered by lack of information transfer and sustained adoption as extension agents are seldom proficient in the technologies themselves to clearly communicate to the farmers who are generally illiterate and operating at subsistence level (Brummett and Costa-Pierce, 2002). However, production of fish from aquaculture, which comprises mainly tilapia, has increased to >500 Mt/year from 1999 to 2001 (GOM-Fisheries Department, 1998; FAO, 2003). Research has concentrated on improving the performance of indigenous species since the government banned the introduction of exotic fish species in 1991. The lack of suitable fish species to

boost aquaculture continues to be one of the challenges in aquaculture development in Malawi (Kaunda *et al.*, 1997; Nyirenda *et al.*, 2000). Currently the species of fish farmed are all indigenous and include *Oreochromis shiranus shiranus*, *Oreochromis shiranus chilwae*, *Tilapia rendalli*, *Clarias gariepinus* and lately *Oreochromis karongae*. *Tilapia rendalli*, considered in my study, is one of the more preferred species grown by smallholder farmers in Malawi.

Generally, *Tilapia rendalli* (Boulenger) belong to the family: *Cichlidae* in the order, *Perciformes* (Perch-like fish), which is the largest order of fishes. *Tilapia rendalli* is native to the Eastern Zaire basin, Lake Tanganyika and Malawi, Cunene, Okavango, Zambezi and east coastal African rivers south of Phongolo (Trewavas, 1982; Hecht and de Moor (no date)). They are found in environments ranging from freshwater to brackish water (Froese and Pauly, 2004; Nelson, 1994) with salinities up to 19‰ and temperature tolerance in natural habitats from 13.5-36.0° C but can tolerate extremes from 11-37° C (Hecht and de Moor (n.d)). It is a predominantly herbivorous fish species. Juveniles feed on plankton while adults feed on macrophytes, algae, insects and crustaceans. Breeding pairs clear vegetation to form a nest and both parents guard the nest. The species has high fecundity in comparison with other cichlids, with approximately 5000-6000 eggs laid at each spawning (Hecht and de Moor (n.d)).

1.2.2 Tilapia and their feeds

Tilapia species have biological differences associated with their breeding habits and anatomical differences associated with their feeding habits. During their early stages, farmed tilapia do not feed on artificial feeds but rely entirely on the natural production of the pond. The adult can be raised solely on natural production in the pond resulting from manuring and/or fertilizing with artificial fertilizers. This natural food can be supplemented with additional foodstuffs (Swift, 1993).

Feeding habits of the tilapiine species are very broad. They can feed on benthic algae, phytoplankton, microphytes, zooplankton, fish eggs, fish larvae and detritus. As tilapia feed low in the food chain, increasing the productivity of the lower trophic level organisms is commonly used to providing adequate natural food in tilapia culture systems (Alceste and Jory, 2000). Tilapia can be fed a variety of feeds including plant leaves, meal and various waste products suitable for other species. Main plants used include cassava, sweet potato, cane, maize and papaw. Waste products include rice bran, cotton seed cake, peanut cake, fruit, brewery waste and coffee pulp. The type of feed used depends on the availability and local cost (Swift, 1993).

Tilapias are opportunistic feeders and will utilize any of these feeds when they are available. This provides an advantage to farmers because the fish can be reared in extensive, semi-intensive as well as intensive systems that can be operated with lower feed costs (Fitzsimmons, 1997). Chikafumbwa (1996a) and Brummett (2000a) reported on terrestrial vegetation and agricultural crop residues having potential in the culture of *T. rendalli* and *O. shiranus* in ponds. *T. rendalli* and *O. shiranus* fed with maize bran and

napier grass (*Pennisetum perperium*) in polyculture, produced good growth and higher yields. This agrees with Philippart and Ruwet (1982) who reported that adult tilapia feed preferentially on filamentous algae, aquatic microphytes and vegetable matter of terrestrial origin. Impressive pond yields have been obtained by feeding only rice bran, brewery waste, copra meal, coffee pulp and animal manure such as poultry, swine and cattle (Alceste and Jory, 2000). Therefore, there are three potential feeding regimes in fish. Firstly, feed may be produced directly in the pond by plants growing in the pond as a result of fertilizing (naturally pond produced). Secondly, feed may be produced in the pond, but because of the high density of the fish population, supplementary food produced outside the pond is provided. Finally food given to the fish is produced outside the pond (complete diets) (NRC, 1993; Swift, 1993; Lovell, 1998). The second regime would be suitable for Malawi to enhance fish production from aquaculture, as the latter would be more expensive and unaffordable by small holder farmers.

1.2.3 Semi-intensive system concept

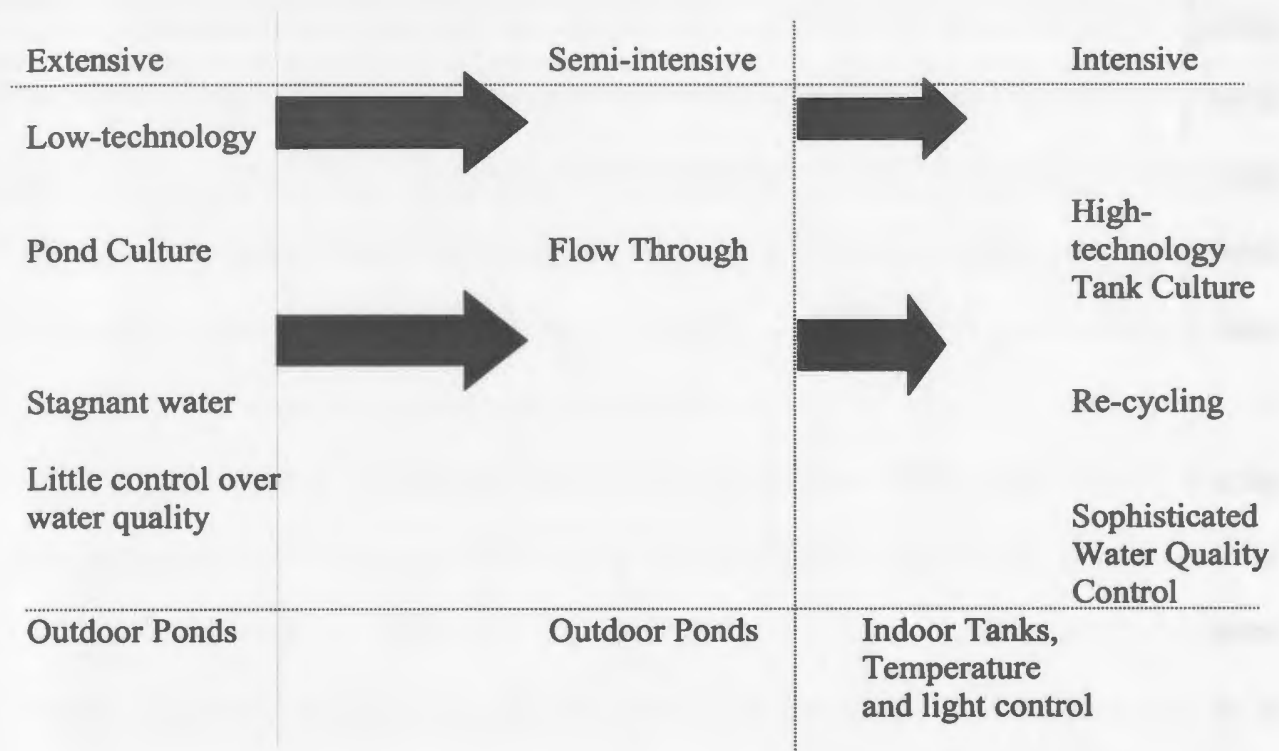
In a semi-intensive system the production of herbivorous and omnivorous fish feeding low on the food chain in ponds is enhanced using fertilizers and supplementary feed (Edwards *et al.*, 2000). The semi-intensive system is characterised by a number of factors: low stocking densities with fish yield ranging from 0.5 to 20 ton/ha/yr as compared to fish yields as high as 1000 ton/ha/yr at high densities in intensive farming systems (Edwards, 1993); the use of lower valued warm water omnivorous/herbivorous fish species within static pond-based farming systems; fish produced mainly for local

consumption; restricted use of traditional fertilizers and/or low cost on-farm prepared supplementary feeds; and, the farming activity being usually small scale in nature and generally integrated within an integrated agriculture-aquaculture farming system (Tacon and De Silva, 1997).

This system is a means of producing low cost fish, which contribute to national food security in many developing countries (Edwards *et al.*, 2000). Semi-intensive aquaculture, particularly in the tropics, accounts for nearly 70% of the finfish cultured in the world (Tacon and De Silva, 1997). Tilapia are amongst the most cultured omnivorous fish produced widely in the world, probably ranking second to carp (*Cyprinus carpio*). Tilapia species have been proposed as one solution to the problem of shortage of fish protein in tropical areas (Pillay, 1993). The schematic presentation of a semi-intensive system in comparison with other production systems is presented below (Fig. 1.1).

Aquaculture production in Malawi is practiced using both extensive and semi-intensive systems. The extensive system is characterised by low stocking rates, low costs, high labour use, and low productivity. In semi-intensive systems, fish are not fed on industrial manufactured feeds, but use manure or organic farm wastes with supplemental feed like maize bran and rice bran (Sikawa, 1998; Brummett, 2000a). A low level of integration is practiced and animals like poultry, pigs, cattle, rabbits and goats are used for both their manure and crop residues, which are put into the ponds.

Fig. 1.1. Flow chart of farming systems (with emphasis on intensification) practiced in aquaculture (Hecht and de Moor (n.d)).



1.2.4 Role of fertilization in food production

To enhance primary production in ponds, fertilization is done using natural (organic) and artificial (inorganic) fertilizers. Organic fertilizers add detritus, which stimulate the heterotrophic food chains thus producing more bacteria and zooplankton. Microphagous tilapias are very responsive to such treatment and yields can be increased considerably (Hepher and Pruginin, 1982; Lovshin, 2000). Artificial fertilizers are expensive compared with organic fertilizers (Swift, 1993). The nature of the organic material and its prior treatment determines the moisture and primary nutrient content of

manure, which varies greatly among the manures. However, manures have much lower percentages of primary nutrients than chemical fertilizers (Boyd, 1982; Knud-Hansen, 1997). Therefore, to ensure optimum production of plankton, regular fertilizing using proper amounts is required. Diana *et al.* (1988) showed that, when male *O. niloticus* in ponds received high fertilizer inputs there were higher nutrient levels in the water, which resulted in higher primary production and higher fish production than ponds treated with low inputs of fertilizers. Their study also revealed that nitrogen and phosphorus appeared to be the limiting factors for primary productivity.

Understanding the carbon and nitrogen ratio is important as a means of reducing the accumulation of inorganic nitrogen in ponds (Avnimelech, 1999). The increase of carbonaceous substrate was found to reduce inorganic nitrogen in shrimp experimental tanks and in tilapia commercial scale ponds (Avnimelech, 1999). Therefore, it is important to note the level of carbon in the organic fertilizers and the supplementary feed used in relation to nitrogen to improve the quality of the water. This also improves the recycling of carbohydrates and increased proteins through the utilization of microbial proteins (Avnimelech, 1998).

The amount of fertilizer required varies with pond fertility and stocking density. Normally application rates are based on weight per unit area of pond surface, kg/ha, kg/m² or number of animals per area (Bocek, 1996; Edwards *et al.*, 1997). Some studies have been conducted on the input of nitrogen and phosphorus/ha/day (Knud-Hansen and Batterson, 1994). A ratio of 4 nitrogen : 1 phosphorus/ha/day, has been encouraged. However, this has been met with mixed reactions because there is an excess of organic

matter that may cause organic matter overloading in the ponds (Edwards *et al.*, 2000). An application of chicken manure to ponds has increased productivity in smallholder ponds, with an optimum level being achieved at a manuring rate of 470 kg/ha/wk. Excessively high inputs, such as 940 kg/ha/wk of poultry manure, have had detrimental effects on fish production due to the deterioration in water quality. Although it is advisable to culture fish which are tolerant to poor water quality, eutrophic conditions may affect such species and cause fish kills (Msiska and Cantrell, 1988; Milstein, 1997). The optimal pond fertilization rate of 4 kg N and 1kg P/ha/day (Knud-Hansen and Batterson, 1994) is as high as those used on most heavily fertilized field crops (ESCAP/FAO/UNIDO, 1991) and may cause environmental concerns (Edwards, 1993). Nitrogen deficiency becomes apparent when ponds are fertilized with less than 500 kg/ha/week (71 kg/ha/day) of chicken manure (Edwards *et al.*, 2000).

In Thailand, the use of pig, cattle and chicken manure in ponds produced yields of up to 4 tons/ha) and with liquid cattle manure and fish in polyculture at high densities, high yields were obtained and replaced supplementary feed to a large extent (Hepher and Pruginin, 1982). The fresh manure in this case disintegrated in the water into colloidal particles that were attacked by bacteria and readily incorporated in the food web. Integrated farming systems, where animals are kept over fish ponds and their wastes fall directly into the pond, usually result in high yields with tilapia (Hepher and Pruginin, 1982). Organic fertilizers may also serve as a direct source of food for invertebrates and fish, or may decompose, releasing inorganic nutrients that stimulate plankton growth. Organic fertilizers are more efficient in increasing the abundance of zooplankton and

benthic organisms such as chironomids than chemical fertilizers. The use of animal manure may be cheaper but has to be carefully controlled by the farmer and must be managed carefully to avoid contamination of the product with animal pathogens, parasites, heavy metals, antibiotics and other substances potentially harmful to consumers (FAO, 1997a).

1.2.5 Use of supplementary feed

Farmed fish obtain their food partly from the natural production in the pond and partly as supplementary feed given by the farmer. Tilapia can be easily fed with numerous plants, farinaceous vegetation and different kinds of waste. Plants are particularly suitable for the herbivorous fish while farinaceous food is satisfactory for all species (Huet, 1994). The principal plants used are leaves of cassava, sweet potatoes, eddoes, banana trees, papaw, maize, canna and legumes. Among the farinaceous foods are generally found meal wastes like cassava bran and flour, chips and balls of rice, corn flour, cotton and groundnut oil cakes. Industrial and domestic wastes such as decomposed fruit, brewery draff, coffee pulp and wastes from local beverages and termites may also be used (Huet, 1994). However, not much information is provided on the effectiveness of these feedstuffs and it seems that some of the feeds are less effective than others (Hepher and Pruginin, 1982).

The supplementary feed acts in two ways; it is used directly as food but also acts as an additional fertilizer (Swift, 1993). The formulated supplementary feeds, however, have had mixed results in terms of the level of formulation. FAO (1994) reported that

most commercial aquafeeds for extensive and semi-intensive pond farming systems are over-formulated as nutritionally complete diets, irrespective of intended fish stocking density and natural food availability, resulting in wastage. In many cases, it is unnecessary and uneconomical to balance diets fed to fish in ponds according to the nutrient requirement of the fish (Lovell, 1980). Tilapias are efficient users of natural food. Most are omnivorous, continuous feeders with the ability to consume planktonic as well as benthic food sources, and because of this they do not require highly formulated diets. Essential factors such as vitamins, trace minerals and amino acids are not of great importance in supplemental pond diets for tilapia (Lovell, 1980). However, tilapia yields with supplementary feeds can be increased 2 to 10 fold over yields from non-fed ponds (Hepher and Pruginin, 1982). Many studies have been done using a variety of feed stuffs in tilapia ponds. Feed development work needs to focus on the efficient use of resources, reduction of feed waste discharge and minimizing the use of fish meal in diets to reduce feed cost and avoid competition with other users (FAO, 2000).

A number of supplementary diets have been tested on tilapia in Malawi and elsewhere. Chimatiro and Costa-Pierce (1996) fed waste vegetable leaves to juvenile *T. rendalli* and *O. shiranus* under polyculture system, with maize bran as a control. Pond inputs had a significant effect on the specific growth rates and weight gain. In vegetable leaf treatments (cabbage and pumpkin leaves), the best weight gains were obtained in monocultures of *T. rendalli* compared to *O. shiranus*. Jamu and Costa-Pierce (1995) found that fish production increased from about 500 kg/ha/yr to 3000 kg/ha/yr in two production cycles using a napier grass/maize bran combination. Maize bran has been the

principal ingredient used in aquaculture in Malawi (Msiska, 1988; Kadongola, 1991; Brummett, 1997). Under a polyculture system (*T. rendalli* and *O. shiranus*), Kadongola (1991) reported that feeding maize bran (madeya) at different feeding rates resulted in elevated fish growth.

Pillay (1993) reported that in a majority of cases, feeds are prepared on the farm using locally available ingredients. Wee and Tuan (1988) reported on the effects of dietary protein level on the growth of *O. niloticus* where complete diets were used. Protein levels of 27.5 to 35% composed of fishmeal, soybean meal, blood meal, corn oil, leucaena leaf meal, cassava starch with vitamin and mineral premixes, were superior to 20% diet. The weight, specific growth rate (SGR), feed conversion efficiency (FCR) and protein efficiency ratio (PER) improved with an increase in protein level. However, in semi-intensive systems, natural food from the pond substantially contributes to the fish growth. Shroeder (1983) reported that a quantitative comparison of the tilapia supplied with pellets or manure and available natural foods, showed that 50 to 70% of the somatic growth of tilapia originated from food-chain based photosynthetic natural foods. This occurred even in the presence of a full ration of protein enriched feed pellets. Thus, low protein diets of 25% or less would be as good as higher protein diets in supplementary diets (Lovell, 1998).

1.2.6 Role of the water environment on growth and feed utilization in tilapia

Many water quality variables may affect a fish's well-being. Fortunately, only a few normally play a decisive role (Boyd and Tucker, 1998). Temperature and salinity are

among the most important factors that influence growth of fish, and play an important role in natural and artificial habitats (Chervinski, 1982; Philippart and Luwet, 1982; Suresh and Kwei Lin, 1992). Understanding the role of temperature and salinity for species in aquaculture would help the fish farmer in the proper selection of a fish species that might perform better in a specific environment. This information could also help expand the utilization of areas of marginal agricultural value to increase food production and income (Kissil, 1996; Likongwe, 2002).

1.2.6.1 Effects of temperature on growth and feed utilization in fish

Temperature is the principal environmental factor that influences the physiology of fish and also affects feeding, metabolism, reproduction and growth. It is the most pervasive and critical environmental variable in the life of fish (Hazel, 1993; Huet, 1994; Martinez-Palacios *et al.*, 1996; Aune *et al.*, 1997; Boyd and Tucker, 1998; Dickerson and Vineyard, 1999; Janassen *et al.*, 1999). In tilapia, temperature has a considerable influence on the vital activities of the fish, notably their respiration, growth and reproduction (Huet, 1994; Ridha *et al.*, 1998).

Variations in culture temperatures are reported to range from 28-30° C for *T. rendalli* and *T. zillii* and 25-30° C for other tilapia, *O. aureus*, *O. mossambicus* and *O. niloticus* (Bocek, 1996). In a study on the effects of temperature on feeding rate and growth in laboratory tanks with red tilapia, Bhikajee and Gobin (1997) found that growth and feeding rates were lowest at ambient temperature (19.9° C) and highest at 32° C, while feed conversion ratios improved with the increase in temperature. The assimilation

rate was better at higher temperatures. Other tropical fish, *Chirostomer estor estor* (Martinez-Palacios *et al.*, 2002), exhibited similar temperature characteristics where 28° C was reported to be the optimum temperature for specific growth rates in juveniles tested under temperatures ranging from 16.1 to 34° C. But it is more difficult to influence the temperature of the pond than it is to improve other means of bettering production, such as fertilization or artificial feeding (Huet, 1994). So temperatures are dynamic and specific for particular fish species.

1.2.6.2 Effects of salinity on growth and feed utilization in fish

Each species of fish has an optimum range of salinity for reproduction and growth and outside this range, performance is diminished and survival may be poor (Boyd and Tucker, 1998). Tilapias are believed to have evolved from a marine ancestor and their penetration into fresh water is secondary (Chervinski, 1982). However, they prefer salinities that promote growth. Some tilapias have a marked euryhaline tolerance to the extent that certain species can grow in saline water after proper acclimation (Suresh and Kwei Lin, 1992; Boyd and Tucker, 1998) (Table 1.1).

Table 1.1 Salinity (‰) range requirements for different species of common tilapia under culture.

Species	Salinity (‰)	Remarks
<i>O. aureus</i>	36-44	Grow well up to salinities of 16-20‰
<i>O. mossambicus</i>	35-40	Spawn and grow well in seawater
<i>O. niloticus</i>	29-35	Grow well in water up to 20‰
<i>Tilapia rendalli</i>	0 -19	Can tolerate brackish water
<i>T. zilli</i>	0 -39	Grow well in full strength sea water
Florida red tilapia	0 -36	Capable of reproduction in seawater (36‰)

Source: Balarin and Hutton (1979); Bocek (1996); Watanabe *et al.* (1997)

The effect of salinity on growth of tilapia has been studied in relatively few species and it is not well understood (Watanabe *et al.*, 1997). An optimum salinity range for growth of *O. aureus* and *O. niloticus* has not been established, but this type of fish can grow better in salinities from 0‰ to 10‰ (Stickney, 1986). At salinities of 23-40‰ *O. aureus* has been found with lesions and reproduction has been adversely affected (Boyd and Tucker, 1998). High tolerance was reported for *O. mossambicus*, which grows and reproduces in both fresh and full strength seawater but prefers brackish water of 10-20‰ (Boyd and Tucker, 1998).

Likongwe (2002) investigated the effect of different salinity concentrations on survival, growth, feed conversion and whole body composition of five taxonomic groups

of tilapia grown in Malawi. Those studied were *O. shiranus chilwae* (Bunda strain), *O. shiranus chilwae* (Lake Chilwa strain), *O. karongae*, *T. rendalli* and *O. shiranus shiranus*. All these except *T. rendalli* and *O. shiranus shiranus* grew better in 10‰. With the exception of *O. shiranus chilwae* (Lake chilwa strain) and *O. shiranus chilwae* (Bunda strain), all species lost carcass protein by the end of the study.

1.3 The problem

Malawi has been experiencing low production from aquaculture with an average annual production of 331.7 tons from 1992-2001 (FAO, 2003). In general, production ranges from 1.2-1.6 ton/ha/yr (Chimatiro and Costa-Pierce, 1996; Sikawa, 1998; Brummett, 2000a). This contrasts with integrated agriculture-aquaculture, which produces between 0.5 to 20 ton/ha/yr (Tacon and De Silva, 1997). However, the provision of feed and feeding products to the farmed fish still remains a challenge to most farmers. The systems are traditional, without the regular use of manure and high quality feed (Noble, 1996). This has led to stagnant growth in production from the smallholder farmers. Little work has been done to improve the status of feed development in many developing countries (Tacon, 1996), including Malawi. Most efforts have been concentrated on traditional methods of keeping the fish, characterised by irregular fertilizing and/or feeding, or letting the fish find their own food once stocked in the pond (Brummett, 1998).

1.4 Purpose of study

In this thesis I address some of the problems that are faced by the fish farmers in Malawi. Nutrition is known to be one of the most important issues of concern that have to be addressed to improve aquaculture production. Malawi has a large surplus of locally available agricultural by-products like rice bran, maize bran, cotton oil cake, soybean cake, sunflower cake, and sugar by-products, which could be used for fish feed (World Bank, 1988). Dispersed small-scale rice and maize mills are now rejecting rice bran as residues, which could be used in rural fish ponds (personal observations). Similarly, livestock distribution also suggests that manure could be used as fertilizers in fish ponds (World Bank, 1988). However, not many farmers utilize these available by-products and manure in their production ponds, which affects their annual fish production.

1.5 Study objectives

The major objective of my study was to develop practical feed and feeding protocols for tilapia (*T. rendalli*) under semi-intensive aquaculture systems in Malawi. To achieve this, the following specific objectives were pursued:

- a) To develop and stimulate natural feed in pond systems through application of different types of locally available organic (manure) fertilizers.
- b) To evaluate affordable low protein supplementary diets that will enhance the growth of fish using the locally available feed ingredients.
- c) To assess the effect of some environmental factors (temperature and salinity) on the growth and feed utilization in fish.

1.6 Experiments conducted

Four experiments were conducted, two in ponds and two under laboratory conditions (Fig. 1.2).

a) The pond experiments were set up to:

1. Determine the effect of using different types of organic animal manure on plankton abundance and growth and survival of *Tilapia rendalli* in ponds.
2. Determine the effect of feeding a single ingredient supplemental feed on plankton abundance, growth, feed utilization and survival of *Tilapia rendalli* in ponds fertilized with chicken manure.

b) Laboratory experiments were set up to:

1. Determine the effect of temperature on growth, feed utilization and survival of *Tilapia rendalli*.
2. Determine the effect of salinity on growth, feed utilization and survival of *Tilapia rendalli*.

The protocols and methodologies used in this research are outlined in the following Chapters and each experiment is covered separately in each Chapter.



Fig. 1.2. Aquaculture facilities (laboratories and ponds) situated at Bunda College of Agriculture, Department of Aquaculture and Fisheries Science, where the study was carried out.

Chapter 2

EFFECT OF DIFFERENT TYPES OF ORGANIC ANIMAL MANURE ON PLANKTON ABUNDANCE AND GROWTH AND SURVIVAL OF *TILAPIA RENDALLI* IN CONCRETE PONDS

2.1 Introduction

The use of manure in aquaculture supports the production of valuable protein using inputs of little nutrient value to man or livestock (Wohlfarth and Hulata, 1987). Animal manures have a long history of use as a source of soluble phosphorus, nitrogen and carbon for algal growth and natural food production (Knud-Hansen, 1998). Animal manure is often used in semi-intensive systems to improve the primary production of the ponds and fish growth (Boyd, 1982; Colman and Edwards, 1987; Mohlfarth and Hulata, 1987; Diana *et al.*, 1988; Msiska, 1988; Knud-Hansen *et al.*, 1993; Edwards *et al.*, 1997; Nguenga *et al.*, 1997; Nwachukwu, 1997). Poultry and cattle manures have been tried with *O. niloticus* and *O. shiranus* in ponds and produced good results (Gupta, *et al.*, 1992; Knud-Hansen *et al.*, 1993; Kamanga and Kaunda, 1998). Pig manure has been tried in aquaculture in many areas (Boyd, 1982; Hepher and Pruginin, 1982). Using pig, cattle and poultry manure, yields of up to 4 ton/ha have been realised (Hepher and Pruginin, 1982). However, Wohlfarth and Hulata (1987) found that with manures as the only organic inputs, there was little difference in yield between ponds fertilized with cattle or poultry manure. Different types of manure have been found to influence the natural productivity of the pond differently in terms of abundance and prevalence of phytoplankton and zooplankton as well as the benthic materials found in ponds. Boyd (1982) reported that poultry manure triggers more production of phytoplankton in ponds than any organic fertilizers including chemical fertilizer.

The availability of such natural food would be beneficial to tilapia under semi-intensive systems with a low or minimum cost of production. Manure has been found to

increase production of fish when utilized efficiently, with appropriate fish stocking densities (Edwards *et al.*, 1988; Edwards *et al.*, 1999) and their frequency of application (Garg and Bhatnagar, 2000). An application rate of cattle and inorganic fertilizers of twice a month gave the highest values of fish biomass, specific growth rates, net productivity, plankton population and nutrient level irrespective of the source of the fertilizer (Garg and Bhatnagar, 2000). Inorganic fertilizers are expensive and their use by smallholder farmers may be limited (Swift, 1993). However, use of organic manure in integrated systems remains poorly developed in many parts of Africa, including Malawi, as opposed to South East Asia where it is well developed (Edwards and Pullin, 1990; Pullin and Prein, 1995).

Traditionally not many fish farmers utilize this cheap resource. This is due to the fact that fish farming is not a primary activity of the farmer in many parts of Malawi (Brummett, 1998). Fish farming is used as a means of supplementing the family income and/or for home consumption (Brummett, 1995; Brummett and Noble, 1995a). Although the World Bank (1988) reported that there is a good number of livestock available that can produce manure for aquaculture in Malawi, the technology is under-utilized and its effect on production of fish in aquaculture remains unexplored. The use of organic manure can be a good option for small-scale farmers because feed may not be always readily available. Inadequate pond input, both quality and quantity, has been identified as one of the key factors limiting production in small-scale aquaculture. The aim of this study was to determine the effect of different types of manure on plankton abundance and growth and survival of *T. rendalli* using chicken, pig and cattle manure.

2.2 Materials and methods

2.2.1 Experimental facilities and set up

This experiment was carried out in ponds at the Bunda College fish farm, a facility of the Department of Aquaculture and Fisheries Science. It was conducted from May to August 2002. Twelve concrete ponds measuring 20 m² (5 m by 4 m) and 1.1 m deep were prepared by laying soil (clay-loam) about 10 cm at the bottom to act as a substrate for the growth of primary production and to simulate an earthen pond. Ponds were filled with water from the nearby dam, which supplies the water to the aquaculture farm. The water was checked to make sure the levels were stable in all ponds before fertilization and stocking of fish. Four treatments; chicken, pig, cattle manure and no-manure as a control were assigned to ponds at random in a completely randomised design (CRD) and each was replicated three times.

2.2.2 Fertilization regime

The cattle manure was obtained from a commercial farm while the poultry and pig manure (Fig. 2.1) were obtained from the Department of Animal Science students' farm. The manure was collected and transported to the fish farm while dry and kept in sacks stacked in the shade. The ponds were fertilized two weeks before the fish were stocked into ponds. This was to make sure that enough production of plankton and other organisms took place. Application rates in this research work were based on earlier research work on fertilization (Hepher and Pruginin, 1982; Gupta *et al.*, 1992; Green and Boyd, 1995; Sikawa, 1998). Application was done once a week by broadcasting at the

following rates: chicken manure at 500 kg/ha/wk, pig manure at 500 kg/ha/wk and cattle manure at 1,200 kg/ha/wk. Higher application rate of cattle manure was due to the fact that nitrogen and phosphorus are lower than in chicken and pig manure. If the day was windy, the manure was mixed in a bucket with water to avoid the wind blowing the manure away while broadcasting.



Fig. 2.1. Samples of organic manure (cattle, pig and chicken) used for fertilizing ponds stocked with *Tilapia rendalli*.

2.2.3 Biochemical analysis of organic manure used

The manures were analysed using standard methods (AOAC, 1990). Analysis of dry matter was done by drying pre-weighed samples in an oven at 105° C for about 16 hours to reach a constant weight, nitrogen analysed using the Kjeldahl method, and phosphorus and potassium analysed using spectrophotometry (Table 2.1).

Table 2.1 Proximate composition (%) of the organic manure applied in experimental ponds (Mean \pm SD).

Proximate component (%)	Organic manure source		
	Chicken	Pig	Cattle
Dry matter (% wet weight)	90.35 \pm 1.65	90.02 \pm 0.11	89.24 \pm 0.71
Nitrogen (% dry weight)	1.23 \pm 0.07	1.20 \pm 0.01	0.69 \pm 0.02
Phosphorus (% dry weight)	1.39 \pm 0.05	1.32 \pm 0.01	0.51 \pm 0.01
Potassium (% dry weight)	0.61 \pm 0.02	0.60 \pm 0.01	0.51 \pm 0.01

2.2.4 Fish stocking and sampling

Juvenile *Tilapia rendalli* of mixed sex were collected from the breeding ponds by seine net. They were kept in a large tank for a one-week acclimation period to make sure the fish were in good condition before stocking. During this time, the fish were fed maize

bran. After one week of acclimation in the holding tank, 40 fish were selected and stocked in each pond at the rate of 2 fish/m² (20, 000 fish/ha). The fish were allowed to acclimate to pond conditions and mortality was monitored. During the acclimation time, dead fish were replaced with fish of similar size. A sample of 20 fish (50% of stocked fish) were weighed and measured for their initial weight and total length and averaged 18.15 ± 2.03 g and 10.42 ± 0.47 cm, respectively. Repeat sampling was done every two weeks using a seine net. The fish were anaesthetised using a benzocaine solution (2 drops/L) when weighing and measuring. Their individual weight (g) and total length (cm) were taken using a balance and a ruler fixed to a measuring board. At the end of the experiment, the ponds were drained and all the fish were counted. These data were used to determine mortality.

2.2.5 Fish growth performance

Fish performance and yield were calculated using the following formulae (NACA, 1985; Watanabe, 1988; Hopkins, 1992):

- a) Weight gain = final mean weight (g) - initial mean weight (g)
- b) Percentage (%) increase in mean weight = $\frac{\text{final mean weight} - \text{initial mean weight}}{\text{initial mean weight}} \times 100$.
- c) Specific growth rate (%/ day) = $\frac{(\log_e \text{ final mean weight (g)} - \log_e \text{ initial mean weight (g)})}{\text{time}} \times 100$.
- d) Gross yield of fish/ha = harvested fish weight (kg)/ unit area (ha)
- e) Net yield of fish/ha = (harvested fish weight - initial fish weight)/unit area (ha)

- f) Survival rate (%) = (initial number of fish - number of dead fish)/ initial number of fish x 100.
- g) Fulton's condition factor = weight (g)/ length³ x 100.

2.2.6 Plankton monitoring and enumeration

Plankton was monitored in all ponds. Zooplankton was enumerated once a week. This was done by passing 50 L of pond water through a nylon plankton collecting net (100 µm). The pond water was collected from 5 different positions around the pond using a 10 L bucket. The samples were then collected in bottles and fixed with 2-4 drops of 10% formalin solution. The organisms were concentrated in a 100 ml centrifuge tube from which sub-samples of 1.0 ml were taken for counting on a Sedgewick-rafter counting chamber mounted on a microscope at 40X magnification (APHA, 1985 and Brummett, 2000a). Individual zooplankton per litre was calculated and categories included copepods, cladocerans (*Daphnia* and *Moina*) and rotifers (*Lecane* and *Brachioni*) using the modified formula from Wetzel and Likens (1991) outlined below:

Number of zooplankton/L = $(C \times V') / (V'' \times V''')$. Where: C = number of organisms counted; V' = volume of the concentrated sample (L); V'' = volume counted (L); V''' = volume of water through which a net was towed (L).

The phytoplankton samples were also collected once a week and quantified as chlorophyll *a* (µg/L) to monitor the level of production in the water. Analysis was done using spectrophotometry by filtering the sample, centrifuging and reading the absorbency (APHA, 1985).

2.2.7 Chemical composition of the fish and zooplankton

A random sample of 20 fish was taken before the start of the experiment. The fish were killed, and weighed before they were dried in an oven. They were then ground and passed through 0.2 mm mesh before being assayed for moisture, crude protein, crude fat, ash and gross energy using standard methods (AOAC, 1990). At the end of the experiment, fish from the various treatments were also assayed for their proximate body composition.

Proximate analysis was performed on zooplankton to determine their chemical composition. At the end of the experiment, zooplankton was harvested from the water before draining during harvesting. Water was drawn from the pond into an improvised zooplankton harvester (Fig. 2.2). The plankton net was fixed to the harvester and water was poured through the net aperture. The zooplankton collected in the plankton collector was then transferred to bigger sampling collection bottles. This was done from each pond as a replicate and later the three replicates were pooled. These samples were then assayed for moisture, crude protein, ash, and gross energy following standard procedures (AOAC, 1990).

2.2.8 Stomach contents analysis

At the end of the experiment, a sample of six fish was taken from each treatment. The fish were dissected and stomachs taken out and stored in 10% formalin solution for later examination of diet. The stomachs were weighed, dissected and the constituent food items separated, enumerated under light microscope and weighed (Hyslop, 1980; Bubinas

and Lozys, 2000; Meschiatti and Arcifa, 2002). Plant fragments were differentiated from detritus on the basis of colour, shape, and cell structure (plants consumed directly are greener and have less surface and marginal distortion and more intact cells than detrital plant material). Differentiation of plankton and detritus was based on subjective indicators such as physical integrity. The stomach contents were grouped as detritus, higher plant, phytoplankton, zooplankton (Brummett, 2000a), insects and others that could not easily be identified. The numerical percentages of the total particles in the stomach content were calculated based on weight (Bubinas and Lozys, 2000).



Fig. 2.2. Zooplankton harvesting in *Tilapia rendalli* ponds fertilized with different types of organic manure using locally made plankton harvester.

2.2.9 Pond sediments analysis

Pond bottom soils were collected once a month during this experiment to assess organic matter loading. A soil sampler was constructed using locally available tins of about 10 cm diameter. A hole was cut at the end to allow water to escape when sampling as in the procedure employed by Brummett (2000a). The cup was pushed down into the sediments at random from two positions in the pond. The organic matter content was determined using the dry ash method (Boyd, 1995). Sediments were dried at 105° C, pulverized, sub-sampled, weighed and ignited in a muffle furnace at 350° C for 8 hours (Ayub and Boyd, 1994). Percent organic matter was estimated by subtracting the weight of the ash from the dry matter.

2.2.10 Water quality monitoring

Temperature (° C), dissolved oxygen (mg/L), pH, electrical conductivity ($\mu\text{S cm}^{-1}$), salinity (‰) were measured using a multi-probe water checker (U-10 model, Horiba Ltd., Japan) by dipping into the water surface. Recordings were taken every day, 7 days a week, at 08:00 and 14:00 hrs throughout the culture period. Secchi disk visibilities (cm), ammonia (mg/L), nitrite (mg/L), total alkalinity as CaCO_3 (mg/L), calcium (mg/L) and phosphorus (mg/L) were also measured once a week using standard methods (APHA, 1985).

2.2.11 Data collection and statistical analysis

The data were collected, stored in notebooks and then entered into the computer in the Excel spreadsheet and exported to the statistical package for analysis. The data were first checked for assumptions of analysis of variance: homogeneity, normality and independence. Where the data were not found to meet the assumptions, transformation was performed accordingly using logarithms and arcsine (Sokal and Rohlf, 1995). The data were then subjected to analysis of variance (ANOVA) using a General Linear Model (GLM), Repeated Measures Design on measurements collected with time. One-way ANOVA was then performed at each time and other calculated data to determine significance. If significant ($P < 0.05$) differences were found in the ANOVA test, Duncan's multiple range test (Duncan, 1955) was used to rank the groups. The data are presented as mean \pm SE or otherwise as stated. All statistical analyses were carried out using SPSS 10.0 (SPSS Inc., 1999).

2.3 Results

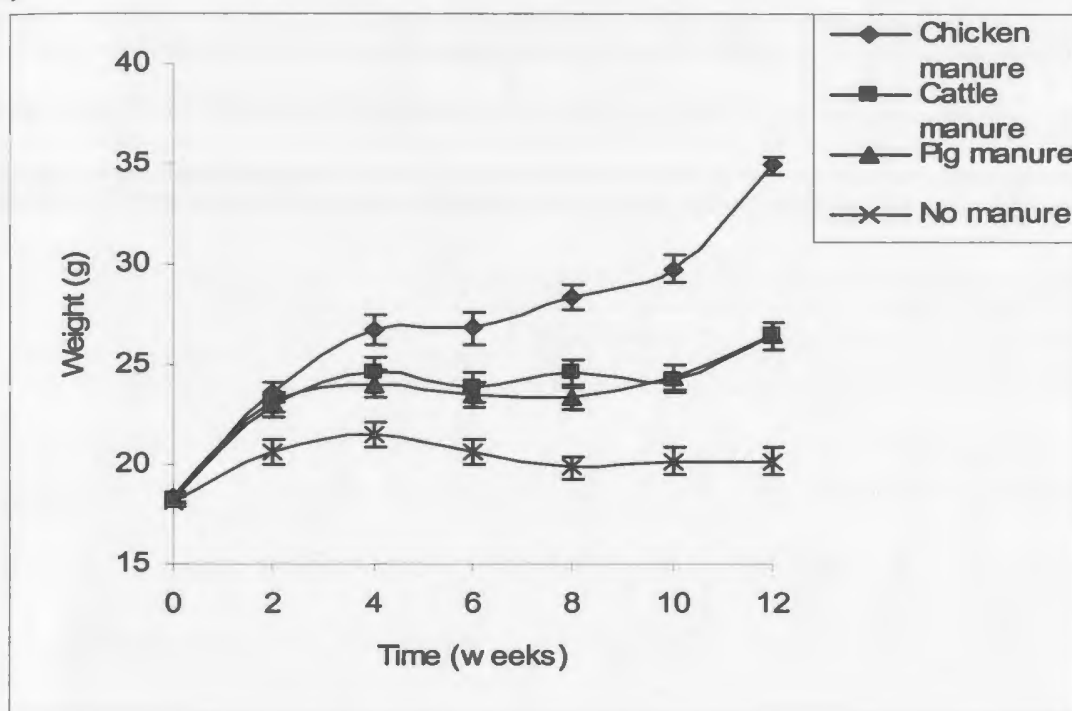
2.3.1 Growth of fish

Data met the assumptions of ANOVA. Fish growth (average weight (g)) in the experiment started showing significant differences ($P < 0.05$) at first sampling, two weeks after stocking (Fig. 2.3). The initial mean weights ranged from 18.14 to 18.38 g and were not significantly different ($P > 0.05$). In the first sampling the no-manure treatment fish had a significantly ($P < 0.05$) lower mean weight (20.66 g) than the rest of the treatments, which ranged from 22.95 g for cattle manure to 23.66 g for chicken manure. Significant differences among the chicken, cattle and pig manure treatments were evident four weeks after stocking with chicken manure producing fish of a significantly higher mean weight (26.71 g). However, during the same period, the mean fish weight for cattle (24.65 g) and pig (24.02 g) manure treatments did not differ significantly ($P < 0.05$). Significant differences among the treatments continued to the end of the experiment, where fish in the chicken manure treatment had significantly higher final mean weights (34.94 g) compared to cattle and pig manure with final mean weights of 26.47 and 26.50 g, respectively. However, fish in the no-manure treatment showed a significantly lower mean weight throughout the experiment with a final mean weight of 20.16 g. The growth of fish in the cattle and pig manure was not significantly different throughout the experiment (Fig. 2.3). Appendix 2.1 provides weight data.

Weight gain followed a similar trend to length of the fish. The fish in the chicken manure treatment had a significantly higher weight gain (16.56 g). The lowest weight gain (2.53 g) was recorded in fish in the no-manure treatment. Weight gain of fish in the

pig (8.27 g) and cattle (8.16 g) manure treatments did not differ significantly. Fish in chicken manure treatment also showed significantly higher specific growth rates (0.77 %/day) than the fish in the other treatments. Specific growth rates of fish in the cattle (0.42 %/day) and pig manure (0.43 %/day) treatments did not differ significantly. However, fish in the no-manure treatment exhibited significantly lower growth rates (0.13 %/day) (Table 2.2). This trend was true also for weight gains per day ranging from 0.03 g/day for no-manure treatment to 0.20 g/day for chicken manure treatment; percentage increase in weight ranged from 15.2% for no-manure treatment to 94.5% for chicken manure. There was a clear trend of increased growth of fish in the chicken manure treatment as compared to the rest of the treatments (Fig. 2.3).

a)



b)

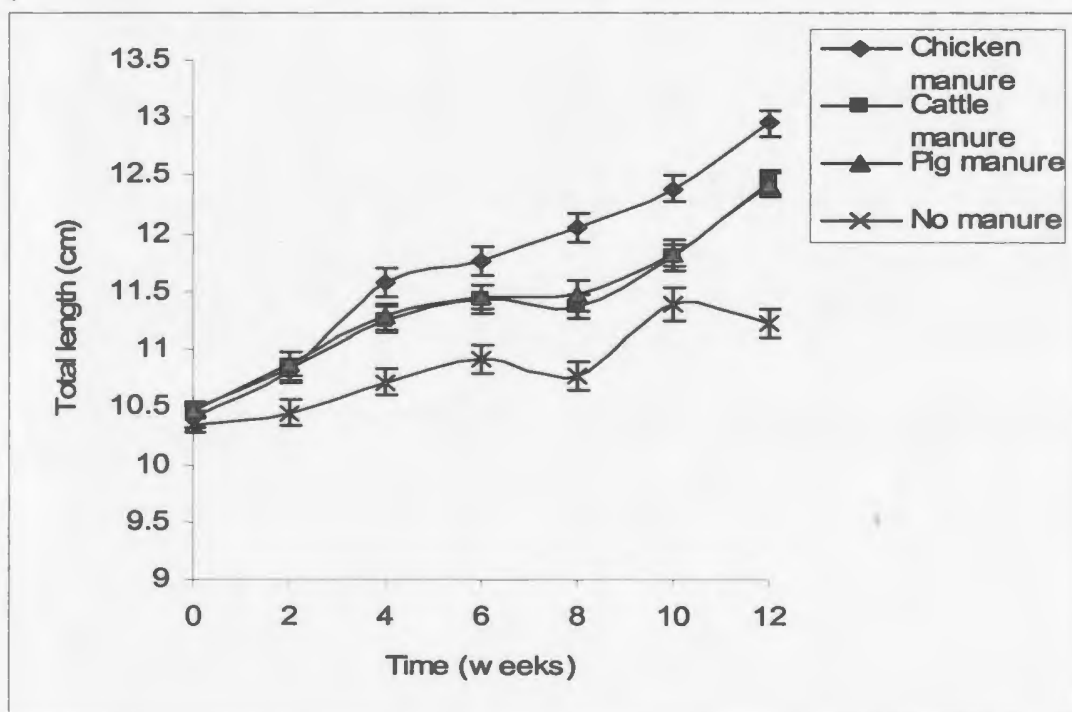


Fig. 2.3. Mean (\pm SE) of weight (a) and total length (b) of *Tilapia rendalli* grown in ponds fertilized with different organic manure (N= 60).

Table 2.2 Initial weight, final weight, weight gain, weight gain per day, weight increase, and specific growth rate (SGR) of *Tilapia rendalli* reared in ponds fertilized with different types of organic manure (Mean \pm SE).

Parameter	Treatment			
	Chicken manure	Cattle manure	Pig manure	No manure
Initial wt (g)	18.38 \pm 0.26	18.31 \pm 0.26	18.23 \pm 0.25	18.14 \pm 0.27
Final weight (g)	34.94 \pm 0.43 ^c	26.47 \pm 0.67 ^b	26.50 \pm 0.66 ^b	20.16 \pm 0.69 ^a
Weight gain (g)	16.56 \pm 0.48 ^c	8.16 \pm 0.78 ^b	8.27 \pm 0.68 ^b	2.53 \pm 0.69 ^a
Weight gain/day	0.20 \pm 0.01 ^c	0.10 \pm 0.01 ^b	0.10 \pm 0.01 ^b	0.03 \pm 0.01 ^a
Weight increase (%)	94.5 \pm 3.6 ^c	47.3 \pm 4.9 ^b	46.7 \pm 4.0 ^b	15.2 \pm 4.0 ^a
SGR (%/day)	0.77 \pm 0.02 ^c	0.42 \pm 0.04 ^b	0.43 \pm 0.03 ^b	0.13 \pm 0.04 ^a

ANOVA: N=240. Initial wt: $F_{3, 236} = 1.743$, $P=0.159$; Final weight: $F_{3, 236} = 95.565$, $P<0.001$; Weight gains: $F_{3, 236} = 75.806$, $P<0.001$; Weight gain/day: $F_{3, 236} = 75.806$, $P<0.001$; Weight increase: $F_{3, 236} = 58.176$, $P<0.001$; SGR: $F_{3, 236} = 58.539$, $P<0.001$.

Note: Values with different superscripts in a row are significantly different (ANOVA, $P<0.05$).

2.3.2 Yield, condition and survival of fish

The yield of *T. rendalli* was affected by type of manure applied. The chicken manure treatment had a significantly higher ($P < 0.05$) gross yield (681.4 kg/ha), net yield (314.8 kg/ha) and annualised net yield (1,255 kg/ha/yr) than other treatments (Table 2.3). Annual net yields produced from cattle (583 kg/ha/yr) and pig (608 kg/ha/yr) manure treatments were not significantly different from each other. Annual net yields from the no-manure treatment (162 kg/ha/yr) were significantly lower than the rest of the treatments (Table 2.3).

Survival was high in this experiment with 97.5% in chicken and pig manure treatments, as well as in no-manure treatment. Cattle manure had a survival rate of 96.7%, which was slightly lower than the rest of the treatments though they were not significantly ($P > 0.05$) different from each other (Table 2.3).

The condition of the fish did not change throughout the experiment. The fish had similar condition factors except for the fish in chicken manure, which had significantly ($P < 0.05$) higher condition factor (1.64%) during the commencement and attained 1.65% at the end of the experiment. Initial condition factors for other treatments were constant at 1.59% but dropped to 1.39%, for both cattle and pig manure treatments; and 1.42% for no-manure treatment (Table 2.3).

Table 2.3 Gross yield, net yield, net annual yield, survival, initial and final condition of *Tilapia rendalli* in ponds fertilized with different organic manure (Mean \pm SE).

Parameter	Treatment			
	Chicken manure	Cattle manure	Pig manure	No manure
Gross yield (kg/ha)	681.4 \pm 8.3 ^c	511.9 \pm 12.9 ^b	516.7 \pm 12.9 ^b	393.1 \pm 13.4 ^a
Net yield (kg/ha)	313.8 \pm 9.4 ^c	145.7 \pm 15.1 ^b	152.1 \pm 13.2 ^b	40.5 \pm 13.5 ^a
Net ann. yield (kg/ha)	1,255 \pm 38 ^c	583 \pm 60 ^b	608 \pm 53 ^b	162 \pm 54 ^a
Survival (%)	97.5 \pm 0.3	96.7 \pm 0.4	97.5 \pm 0.4	97.5 \pm 0.6
Initial condition	1.64 \pm 0.02 ^b	1.59 \pm 0.02 ^a	1.59 \pm 0.01 ^a	1.59 \pm 0.01 ^a
Final condition	1.65 \pm 0.05 ^b	1.39 \pm 0.04 ^a	1.39 \pm 0.04 ^a	1.42 \pm 0.04 ^a

ANOVA: N=240. Gross Yield: $F_{3, 236} = 96.259$, $P < 0.001$; Net yield: $F_{3, 236} = 75.673$, $P < 0.001$; Net ann. yield: $F_{3, 236} = 75.673$, $P < 0.001$; Arcsin Sqrt Survival: $F_{3, 236} = 0.099$, $P = 0.958$; Initial condition: $F_{3, 236} = 3.410$, $P < 0.018$; Final condition: $F_{3, 236} = 9.112$, $P < 0.001$.

Note: Values with different superscripts in a row are significantly different (ANOVA, $P < 0.05$)

2.3.3 Fish body composition

The type of manure affected the whole body composition of the fish in this experiment. Moisture, ash, fat, protein and gross energy were significantly ($P < 0.05$)

different among treatments (Table 2.4). These were also compared with initial composition of the fish before the commencement of the experiment.

Moisture levels were lowest in the initial sample (64.08%) and in the no-manure (64.31%) treatment and did not differ significantly ($P < 0.05$) from each other. Fish cultured in the pig manure treatment had significantly higher moisture than fish in no-manure treatment and those in the initial sample. Body moisture content of fish cultured in chicken (66.84%) and cattle (67.24%) manure were the highest and did not differ significantly from each other (Table 2.4).

Ash content of fish was significantly higher in the initial sample (14.15%) followed by fish in the chicken manure treatment (11.26%). Ash content of fish cultured in the cattle and pig manure treatments did not differ significantly from each other. Overall, the ash content decreased in all treatments compared to the initial composition (Table 2.4).

Fat content differed among the treatments with significantly higher levels in fish cultured in chicken manure (15.94%) followed by pig manure (15.63%), cattle manure (15.32%) and lastly the no-manure treatment (15.04%). The trend shows that there was an increase in fat content from the initial sample (9.22%) (Table 2.4).

The protein content of the fish also significantly differed among treatments. Fish from the no-manure treatment had the lowest protein content (62.97%). The protein content in fish cultured in pig manure (66.50%) and the initial sample (66.68%) did not differ significantly. However, fish cultured in chicken and cattle manure had the highest

protein content (70.51 and 67.86%, respectively) but were not significantly different from each other (Table 2.4).

The type of fertilizer applied also affected gross energy content of the fish. Initial fish energy composition was significantly lower (18.4 kJ/g) than in fish cultured in cattle (25.0 kJ/g), pig (25.0 kJ/g) and the no-manure (22.5 kJ/g) treatments, which were not significantly different from each other. A significantly higher gross energy level (32.1 kJ/g) was found in fish cultured in chicken manure ponds. The percent increases in gross energy content from the initial energy level of 18.4 kJ/g to 22.5 kJ/g in no-manure, 25.0 kJ/g in cattle, 25.0 kJ/g in pig, and 32.1 kJ/g in chicken manure were 23, 37, 37, and 74%, respectively (Table 2.4). The fish in the chicken manure treatment gained a lot of energy in their body compared to the rest of the treatments.

2.3.4 Plankton abundance

The type of manure applied significantly influenced plankton production in the fish ponds. There were significant differences in numbers among the classes of zooplankton such as copepods, cladocerans and rotifers. The phytoplankton, quantified by analyzing chlorophyll *a*, was also significantly affected by treatments.

Copepod numbers per liter of water sampled were significantly ($P < 0.05$) different among treatments. The no-manure treatment had significantly lower numbers of copepods (4,963/L) compared to cattle (22,364/L), chicken (24,140/L) and pig manure (26,922/L) which did not differ significantly from each other. This indicated that the influence of chicken, cattle and pig manure on copepod propagation was similar despite the differences in number of copepods recorded (Fig 2.4).

Table 2.4 Whole body composition (moisture, ash, crude fat, crude protein and gross energy) of *T. rendalli* in ponds fertilized with different organic manure (dry weight) (Mean \pm SE).

Treatment	Proximate Component				
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Energy (kJ/g)
Initial	64.08 \pm 0.29 ^a	14.15 \pm 0.25 ^d	9.22 \pm 0.08 ^a	66.68 \pm 0.30 ^b	18.4 \pm 0.14 ^a
Chicken manure	66.84 \pm 0.08 ^c	11.26 \pm 0.06 ^c	15.94 \pm 0.03 ^c	70.51 \pm 0.21 ^c	32.1 \pm 0.09 ^c
Cattle manure	67.24 \pm 0.25 ^c	9.55 \pm 0.05 ^b	15.32 \pm 0.07 ^c	67.86 \pm 0.13 ^c	25.0 \pm 0.16 ^b
Pig manure	65.35 \pm 0.31 ^b	9.63 \pm 0.04 ^b	15.63 \pm 0.08 ^d	66.50 \pm 0.26 ^b	25.0 \pm 0.09 ^b
No manure	64.31 \pm 0.11 ^a	9.01 \pm 0.07 ^a	15.04 \pm 0.06 ^b	62.97 \pm 0.09 ^a	22.5 \pm 0.09 ^b

ANOVA: N=39, Moisture: F_{4, 34} = 37.969, $P < 0.001$; Ash: F_{4, 34} = 514.051, $P < 0.001$; Fat: F_{4, 34} = 803.476, $P < 0.001$; Protein: F_{4, 34} = 214.924, $P < 0.001$; Energy: F_{4, 34} = 1444.132, $P < 0.001$.

Note: Values with different superscripts in a column are significantly different (ANOVA, $P < 0.05$)

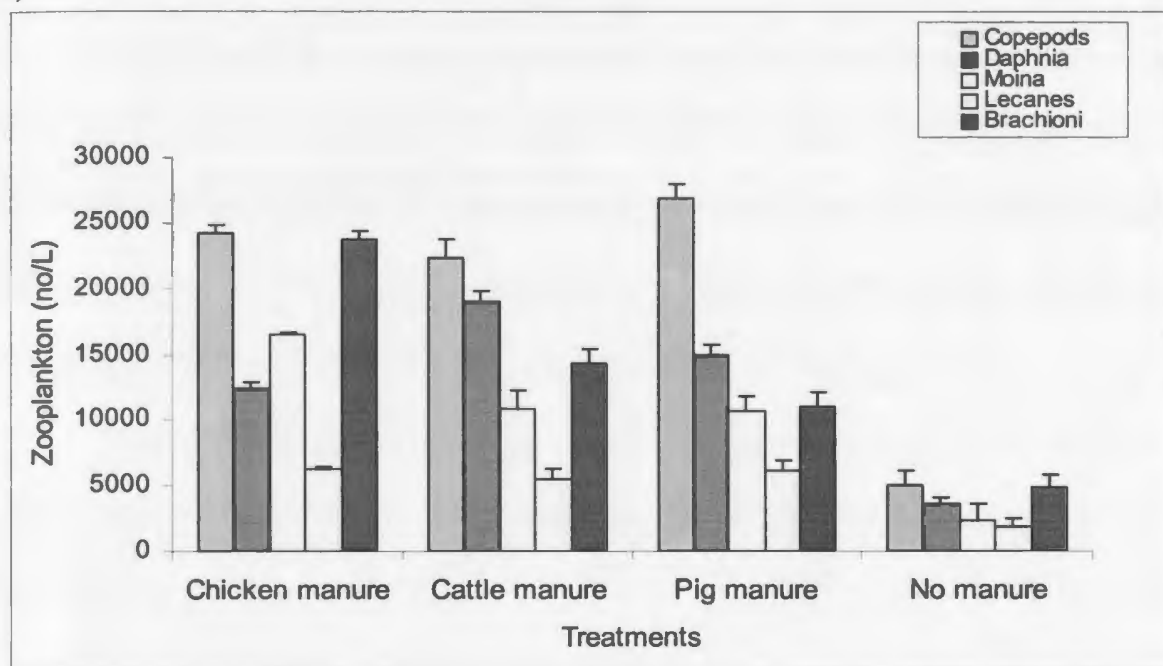
The type of manure applied influenced the cladocerans, which were separated into *Daphnia* and *Moina* genera. Cattle manure propagated significantly higher numbers of *Daphnia* (19,060/L) compared to pig manure (14,855/L) and chicken manure (12,453/L). But *Daphnia* numbers in chicken and pig manure did not differ significantly ($P < 0.05$) from each other. The no-manure treatment propagated significantly lower numbers of

Daphnia (3,548/L). As far as *Moina* was concerned, the propagation was highest in chicken manure (16,457/L), with no significant differences found between the cattle (10,875/L) and pig manure (10,723/L) treatments. The lowest numbers of *Moina* (2,362/L) were propagated in no-manure treatments. Appendix 2.2 provides numbers per litre of zooplankton.

Rotifer propagation was divided in two genera, *Lecanes* and *Brachioni*. These were also significantly influenced by the treatments. The no-manure treatment produced significantly ($P < 0.05$) lower numbers of *Lecane* (1,817/L) than the rest of the treatments. But *Lecane* numbers did not differ significantly among the chicken, cattle and pig manure treatments. The *Brachioni* rotifers did not follow the trend of the *Lecanes*. Significantly ($P < 0.05$) higher numbers of *Brachioni* were propagated in the chicken manure treatment (23,649/L) compared to the cattle (14,267/L) and pig manure (11,042/L) treatments, which did not differ significantly from each other. Significantly lower numbers of *Brachioni* species were found in the no-manure treatment (4,945/L) (Fig. 2.4).

Phytoplankton production in the form of chlorophyll *a* indicated that chicken manure treatment propagated significantly higher amounts of chlorophyll *a* (94.9 µg/L) than cattle (72.9 µg/L) and pig manure (70.7 µg/L), which did not differ significantly from each other. The no-manure treatment had a significantly lower amount of chlorophyll *a* (30.4 µg/L) (Appendix 2.2).

a)



b)

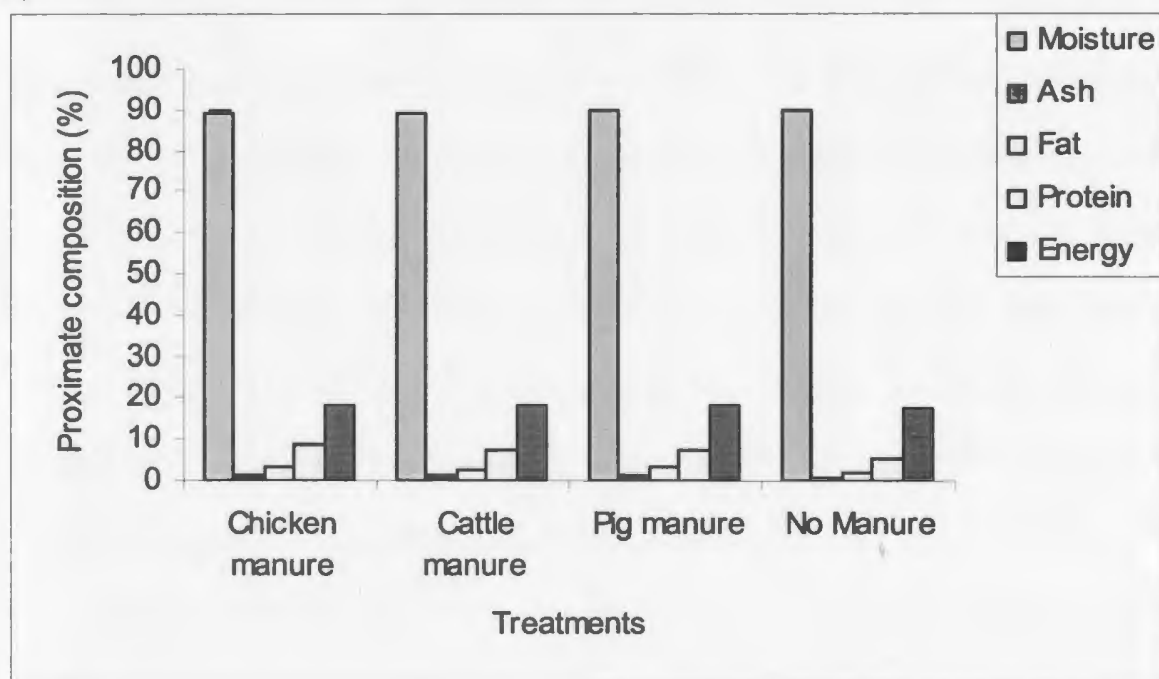


Fig. 2.4. Mean (\pm SE) of population sizes of different classes of zooplankton (a) and their proximate components (b) in ponds fertilized with different organic manure and stocked with *Tilapia rendalli*.

2.3.5 Proximate composition of zooplankton

The analysis of moisture, ash, fat, protein and gross energy of the zooplankton harvested in ponds showed that the manure application had a significant effect on their body composition with the exception of moisture content. Moisture content did not differ significantly ($P > 0.05$) among the treatments, ranging from 89.32% for the cattle manure treatment to 89.59% in the no-manure treatment (Fig. 2.4; Appedix 2.3).

The ash content of zooplankton differed significantly ($P < 0.05$) among the treatments and was highest in zooplankton in the chicken manure treatment (1.5%) followed by cattle manure (1.39%), pig manure (1.21%) with the lowest ash content in the no-manure treatment (0.85%)(Fig. 2.4).

Fat content also differed significantly among the treatments. The highest fat levels were found in zooplankton from the pig manure (3.41%) treatment followed by the chicken (3.27%) and cattle manure (2.46) and lastly the no-manure treatment (1.94%).

The protein levels in zooplankton also differed significantly among the treatments. The highest protein levels were found in zooplankton from the chicken manure treatment (8.46%) and the lowest levels were in the no-manure treatment (5.10%). The protein levels of zooplankton from cattle manure (7.29%) and pig manure (7.24%) did not differ significantly from each other.

Energy followed the trend of fat content and differed significantly among treatments. Significantly ($P < 0.05$) higher gross energy was found in zooplankton sampled from the chicken manure treatment (18.4 kJ/g) with the lowest energy found in zooplankton from the no-manure treatment (17.3 kJ/g). The energy content of

zooplankton from the cattle (18.0 kJ/g) and pig manure (18.1 kJ/g) fertilized ponds was not significantly different from each other (Fig. 2.4)(Appendix 2.3).

2.3.6 Stomach contents

The stomach content of the fish was variable and depended on the type of manure used. Detritus was commonly found in stomachs of fish cultured in the no-manure treatment. The fish had a significantly ($P < 0.05$) higher amount of detritus (51.1%) in their stomachs followed by fish cultured in pig manure (41.1%), cattle manure (39.1%) and lastly those in chicken manure, which had a significantly lower amount of detritus (17.7%). The in detritus content in the stomachs of fish cultured in cattle and pig manure were not significantly different from each other (Fig. 2.5).

Higher plant content in fish stomachs had significant differences among treatments. Plant material was predominant in fish stomachs from chicken manure treated ponds (29.1%) and was significantly higher than cattle manure (15.4%), no-manure (12.7%) and pig manure treatments (7.1%).

Zooplankton was significantly ($P < 0.05$) higher in stomachs of fish from chicken manure treated ponds (16.6 %) followed by pig manure (15.0%) and cattle manure (12.8%). The lowest amount of zooplankton was found in stomachs of fish in the no-manure treatment (10.5%).

Phytoplankton in the stomachs of fish differed significantly among the treatments. Fish stomachs from pig manure treated ponds had significantly higher amount of phytoplankton (28.7%) followed by chicken manure (27.3%) and cattle manure (24.4%).

The lowest amounts was of phytoplankton found in stomachs of fish from the no-manure treatment (15.9%) (Fig 2.5).

Insects were significantly ($P < 0.05$) higher in stomachs of fish cultured in the no-manure ponds (3.8%) followed by chicken manure (1.9%), cattle manure (0.7%) and lastly the pig manure treatment (0.2%). However, there was no significant difference between the cattle and pig manure treatments. The "other" category of stomach contents, which could not be clearly identified was consistent among treatments and did not differ significantly ($P > 0.05$) although the no-manure treatment showed a slightly lower percentage of "other" than the rest of the treatments. Insects were not consumed in large amounts but detritus was highly consumed across treatments (Fig 2.5). Appendix 2.4 provides stomach contents data.

2.3.7 Organic matter loading

The percent organic matter loading was significantly ($P < 0.05$) different among treatments with the no-manure treatment significantly lower (0.48%). However, organic matter loading did not differ significantly among chicken (6.68%), cattle (8.25%) and pig (7.76%) manure fertilized ponds despite the fact that cattle manure was applied at a higher rate (Table 2.5). The calculated nutrient input of nitrogen, phosphorus and potassium per week provided different levels depending on the type of manure and amount of manure applied.

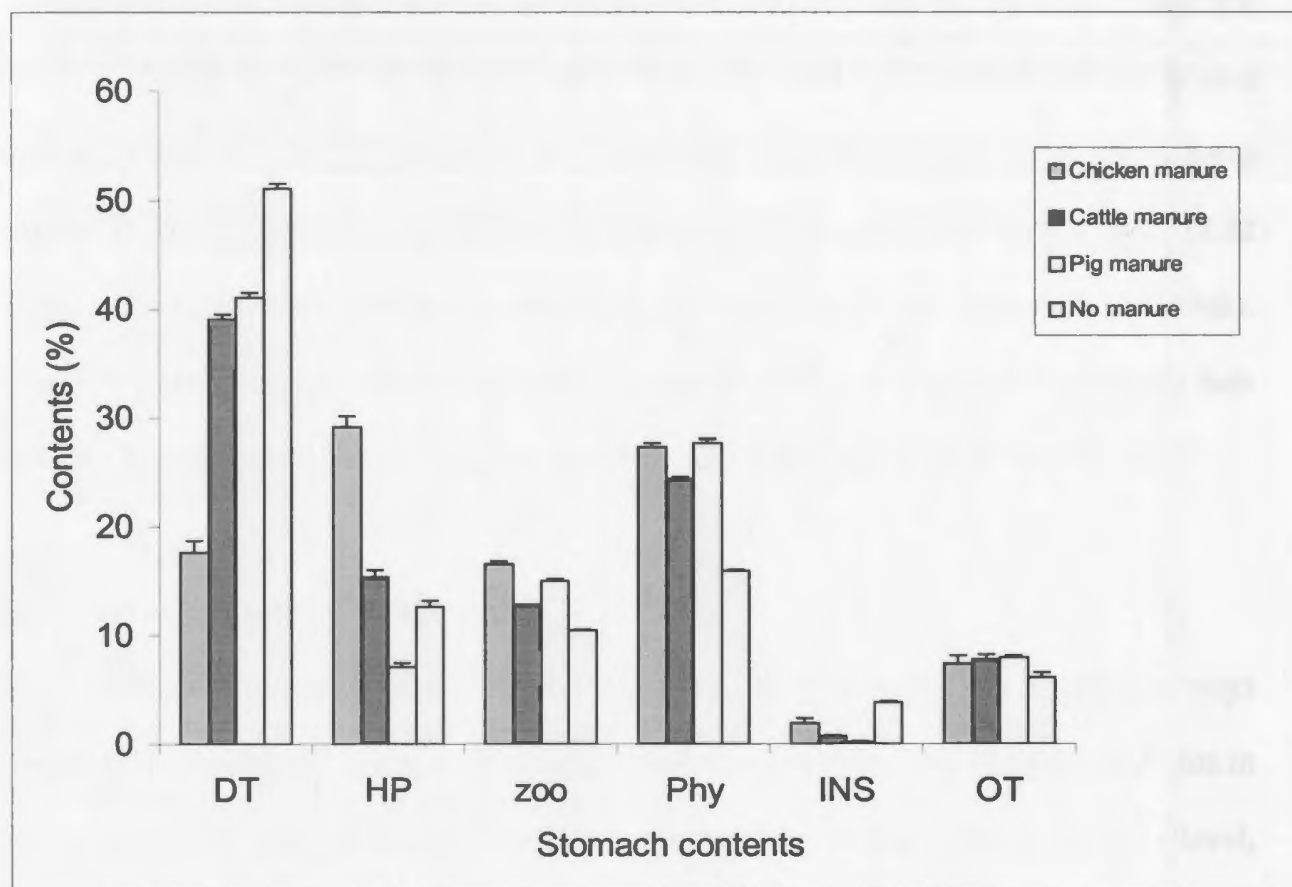


Fig. 2.5. Mean (\pm SE) of stomach contents, detritus (DT), higher plants (HP), zooplankton (ZOO), phytoplankton (PHY), insects (INS) and others (OT) of *Tilapia rendalli* in ponds fertilized with different organic manure.

The nutrient levels corresponded to the application rates and the amount of nutrient present in the manure. The total amount of chicken and pig manure applied during this experiment was the same, 36 kg, and 86 kg for cattle manure, an application rate 2.4 times higher than the other manure rates. Nitrogen input per hectare per week was relatively high in cattle (8.04 kg/ ha/wk) compared to chicken (6.15 kg/ha/wk) and pig manure (6.00 kg/ha/week). Phosphorus input level was also relatively high in cattle (7.32 kg/ha/wk) compared to chicken manure (7.00 kg/ha/wk) and pig manure (6.50 kg/ha/wk). Potassium followed the same trend with the highest rate in cattle (6.24 kg/ha/wk) then chicken 3.05 kg/ha/wk) and finally pig manure (3.00 kg/ha/wk) (Table 2.5, Fig. 2.6).

2.3.8 Water quality parameters

The manure application affected the quality of the water in a number of ways during this experiment. There were significant differences ($P < 0.05$) among treatments in pH, conductivity, morning and afternoon oxygen levels, ammonia, nitrite, alkalinity level, secchi disk visibilities, turbidity as well as phosphorus and potassium. However, there were no significant differences in morning and afternoon temperatures and salinity levels (Table 2.6). The pH of the water was significantly ($P < 0.05$) lower in the chicken manure treated ponds (7.63) and significantly higher levels were recorded in the no-manure treatment (7.91). However, pH did not differ significantly in the cattle manure (7.79) and pig manure (7.80) treatments (Table 2.6).

Table 2.5 Application rate, total manure, organic matter load (OM load), calculated nitrogen, phosphorus and potassium load in ponds cultured with *Tilapia rendalli* and fertilized with different types of organic manure.

Parameter	Treatment			
	Chicken manure	Cattle manure	Pig manure	No manure
Application rate (kg/ha/wk)	500.00	1, 200.00	500.00	-
Total manure (kg)	36.00	86.40	36.00	-
Nitrogen (kg/ha/wk)	6.15	8.04	6.00	-
Phosphorus (kg/ha/wk)	7.00	7.32	6.50	-
Potassium (kg/ha/wk)	3.05	6.24	3.00	-
OM load (%)	6.68 ^b	8.25 ^c	7.76 ^{bc}	0.48 ^a

ANOVA: OM load: N=72. $F_{3,68} = 72.134$, $P < 0.001$

Note: OM load only statistically tested and values with different superscripts in a row are significantly different (ANOVA, $P < 0.05$).

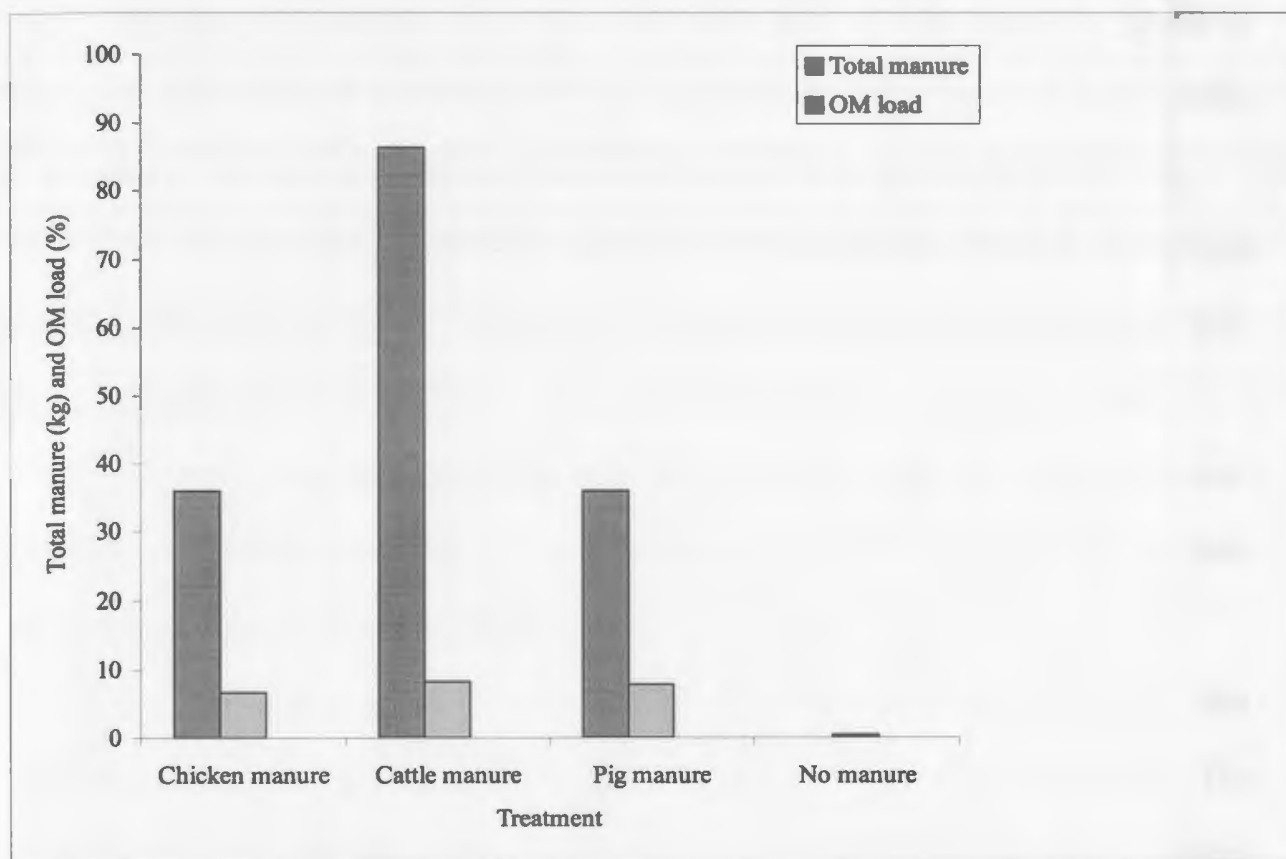


Fig. 2.6. Relationship between organic manure applied (kg) and organic matter loading (OM load) (%) in ponds stocked with *Tilapia rendalli* fertilized with different organic manure.

Dissolved oxygen levels were lower in the morning, ranging from 0.3 mg/L in the chicken manure treatment to 3.4 mg/L in the no-manure treatment. This experiment was conducted during the cool season in Malawi and the temperatures were low during this time (18.4 –18.5° C). However, there were no significant ($P > 0.05$) differences in the temperatures across treatments. The conductance of the water differed significantly among treatments. The no-manure treatment had significantly lower conductance (366 μ S

cm⁻¹) while the highest conductance was in the cattle manure treatment (438 $\mu\text{S cm}^{-1}$). The conductance in chicken (393 $\mu\text{S cm}^{-1}$) and pig manure (394 $\mu\text{S cm}^{-1}$) did not differ significantly. Salinity levels did not differ significantly among treatments (Table 2.6).

Ammonia and nitrite levels in water did not differ significantly among treatments although lowest levels were recorded in the no-manure treatment (0.17 mg/L and 0.01 mg/L, respectively).

The secchi disk visibilities were significantly higher in the no-manure treatment. However, secchi disk visibilities did not differ significantly ($P < 0.05$) among the chicken, cattle and pig manure treatments (Table 2.6).

The relationships between secchi disk visibilities and chlorophyll *a* showed that there was an increase in chlorophyll *a* with a decrease in secchi disk visibilities. The trend was the same in most of the treatments except for the no-fertilization treatment where the secchi disk visibilities remained highest and chlorophyll *a* levels remained relatively low throughout the experimental period. Appendix 2.5 shows the relationship between secchi disk visibilities and chlorophyll *a*.

The dynamic relationship between temperature and dissolved oxygen is important. The temperature readings were consistent throughout the experiment. The morning dissolved oxygen levels were lower in manure treated ponds than in the no-manure treatment. The no-manure treatment had higher morning dissolved oxygen levels than the other treatments. It was also noted that after about 30 days the morning dissolved oxygen increased in the no-manure treatment as compared to the other treatments.

Appendix 2.6 provides the relationship between temperature and dissolved oxygen over the experimental period.

2.4 Discussion

Fish in ponds fertilized with chicken manure grew significantly better than fish in the other treatments. This result is consistent with fertilization systems where yields are above those from unfertilized ponds (Edwards *et al.*, 1988). The specific growth rates (%/day) of fish in this study were significantly higher in chicken manure treated ponds. The growth rates were similar to those commonly found in semi-intensive systems where organic manures are used. Chikafumbwa *et al.* (1993) reported low growth rates for *T. rendalli* (0.42 %/day) and *Oreochromis shiranus* (0.37 %/day) in ponds supplied with napier grass, *Pennisetum perpureum*. These were comparably lower than the present experiment where the fish in chicken manure had specific growth rates of 0.77 %/day. However, their results compare well with the specific growth rates in the cattle manure (0.42 %/day) and pig manure (0.43 %/day) treatments reported in this experiment.

In another study, Chikafumbwa (1996a) reported high growth rates in *T. rendalli* supplied with whole and chopped napier grass attaining 1.20 %/day and 1.29 %/day, respectively. Chaula *et al.* (2002) reported 0.70 %/day for *O. shiranus* in a similar input system, which was within the range of the present results, though slightly lower. Garg and Bhatnager (2000) reported similar specific growth rates (0.71 %/day) in Indian major carp, *Cirrhinus mrigalla*, grown in ponds fertilized with a mixture of cow dung, triple superphosphate and urea.

Table 2.6 Water quality parameters measured in ponds fertilized with different organic manure and stocked with *Tilapia rendalli* for 84 days (Mean \pm SE).

Parameter	Treatment			
	Chicken manure	Cattle manure	Pig manure	No manure
pH	7.63 \pm 0.02 ^a	7.79 \pm 0.02 ^b	7.80 \pm 0.02 ^b	7.91 \pm 0.02 ^c
Conduct. (μ S cm ⁻¹)	393 \pm 3.1 ^b	438 \pm 4.3 ^c	394 \pm 3.5 ^b	366 \pm 3.1 ^a
Oxygen (mg/L mean)	7.8 \pm 0.2 ^a	8.1 \pm 0.2 ^{ab}	8.4 \pm 0.2 ^b	9.3 \pm 0.1 ^c
Oxygen (mg/L range)	0.3 -19.9	1.1 - 19.9	0.4 -19.9	3.4 -19.9
Temp (° C mean)	18.4 \pm 0.1	18.5 \pm 0.1	18.4 \pm 0.1	18.5 \pm 0.01
Temp (° C range)	13.8-25.4	13.8 - 25.8	13.6 - 26.0	13.6- 26.1
Salinity (‰)	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0
Ammonia (mg/L)	0.22 \pm 0.01	0.23 \pm 0.01	0.23 \pm 0.01	0.17 \pm 0.01
Nitrite (mg/L)	0.02 \pm 0.0	0.02 \pm 0.0	0.02 \pm 0.0	0.01 \pm 0.0
Alka-CaCO ₃ (mg/L)	50.8 \pm 1.02 ^d	43.1 \pm 0.87 ^c	40.4 \pm 0.55 ^b	33.1 \pm 0.35 ^a
Phosphorus (mg/L)	0.21 \pm 0.0 ^c	0.20 \pm 0.0 ^b	0.20 \pm 0.0 ^b	0.19 \pm 0.0 ^a
Calcium (mg/L)	9.37 \pm 0.13 ^d	6.63 \pm 0.13 ^c	5.49 \pm 0.08 ^b	3.72 \pm 0.18 ^a
Secchi disk (cm)	27.2 \pm 0.9 ^a	26.0 \pm 0.8 ^a	25.4 \pm 0.8 ^a	39.0 \pm 0.3 ^b

ANOVA. N=1008. pH: $F_{3, 1004} = 69.204$, $P < 0.001$; Conductivity: $F_{3, 1004} = 69.204$, $P < 0.001$; Oxygen-mean: $F_{3, 1004} = 15.393$, $P < 0.001$; Temperature-mean : $F_{3, 1004} = 0.235$, $P = 0.872$; Salinity: $F_{3, 1004} = 0.851$, $P = 0.468$.; Ammonia, N=144: $F_{3, 140} = 0.243$, $P = 0.867$; Nitrite: $F_{3, 140} = 1.113$, $P = 0.346$. Alkalinity: $F_{3, 140} = 8.249$, $P < 0.001$; Phosphorus: $F_{3, 140} = 5.533$, $P < 0.001$; Calcium: $F_{3, 140} = 9.693$, $P < 0.001$; Secchi disk: $F_{3, 140} = 2.894$, $P = 0.034$.

Note: Values with different superscripts in a row are not significantly different (ANOVA, $P > 0.05$).

In a slightly different situation, Middendorp (1995) reported negative growth rates (-0.2 %/day in males and -0.3 %/ day in females) for *O. niloticus* raised in polyculture with catfish, *Clarias gariepinus* in ponds fertilized with cattle manure. However, these were adult fish, which were stocked at an average initial weight of 224 g compared to the initial weight of about 18 g in the present experiment. This would be expected when the carrying capacity is reached and food is not available in the pond. It might be that the cattle manure could not generate enough natural food to keep up with fish growth.

The percent increase in weight for *T. rendalli* was higher for fish in chicken manure (94.5%) in this experiment compared to those from similar systems where a 65% increase was reported for fish in ponds treated with bamboo trunks and poultry manure. Ponds fertilized with poultry droppings resulted in a 37.5% increase in weight for *T. zilli* (Nwachukwu, 1997). Cattle and pig manure treatments produced even higher increases in weight (47.3% and 46.7%, respectively) in this experiment compared to poultry manure alone (Nwachukwu, 1997). Fish in chicken manure ponds performed better than fish in cattle manure in pond trials reported by Brooks and Maluwa (1997). They suggested, however, that cattle manure should not be ruled out as an option for pond fertilization as application rates can be increased to match similar nutrient loading of chicken manure. This is consistent with the approach taken in the present experiment, which improved the survival and growth of *T. rendalli*.

The yield of fish from the chicken manure treatment was significantly higher than from the rest of the treatments. Yields in Sub-Saharan Africa typically range from 100-500 kg/ha/yr in low input, low output systems (Hecht and de Moor (n.d)). The yields in

my experiment were higher than 500 kg/ha/year in all treatments except for the no-manure treatment. My extrapolated net yields (kg/ha/yr) from chicken manure (1,255 kg/ha/yr) were within the range of those reported by earlier researchers in Malawi (Costa-Pierce *et al.*, 1991; Chikafumbwa *et al.*, 1993, Brummett and Noble, 1995b). Brooks *et al.* (1997) reported *T. rendalli* raised in ponds with fertilization produced net yields ranging from 1000-1,500 kg/ha/yr. The typical production of smallholder farmers under project conditions ranged from 1,180 to 1,440 kg/ha/yr (Dickson *et al.*, 1997). The present yields are higher than reported by Chaula *et al.* (2002) which was 932 kg/ha/yr for *O. shiranus* grown in ponds supplied with napier grass at 350 kg/ha/wk plus urea (25 kg/ha/wk). Their results however, were higher than the yield obtained in the cattle and pig manure treatments in the present experiment. Juliano (1985) reported even lower yields in milkfish, *Chanos chanos*, 767 kg/ha/yr with chicken manure in the Philippines. In similar systems with same species *C. chanos*, Bombeo-Tuburan *et al.* (1989) reported gross yields of 1,317 kg/ha/yr, which were comparable to yields obtained in the present experiment.

The survival rates in the present experiment were higher than reported by Chikafumbwa (1996a) and did not differ among treatments. There was no sign of fish reaching carrying capacity although differences in average weight were found in the first sampling. The fish showed continuous growth, though slow, due to low temperatures during the experimental period. However, Brooks and Maluwa (1997) reported low survival in the cool season production cycles with *T. rendalli* as compared to the warmer season in pond trials in Malawi. They suggested that stocking fish of 10-20 g could

increase survival in the cool season, as was the case in the present experiment where the initial average weight of the fish was about 18 g. This might have improved the survival rate as suggested by Brooks and Maluwa (1997).

Proximate analysis of the fish for moisture, ash, fat, protein and gross energy indicated that fish in the chicken manure treatment had significantly higher levels for most of the proximate components. Application of manure had a significant effect on the proximate composition of the fish indicating that the availability of food, among other factors, could influence the composition. The amount of protein in the present experiment is within the range reported by Yi *et al.* (2002) where they fed different species of phytoplankton to sex reversed Thai red tilapia. However, they reported higher moisture (83%) and ash (23-26%) content in their study than reported in this experiment. This may be due to the species and saline environment in their experiment. Veverica *et al.* (2000) in inorganic fertilized ponds stocked with *T. niloticus* and *C.gariepinus*, reported a decrease in fat content and a slight increase in moisture, protein and ash. This was in contrast to fairly high increases in fat content and a reduction in ash from the initial samples experienced in the present experiment across treatments.

The natural plankton production data indicated that chicken manure had a significant influence on both phytoplankton and zooplankton. Significantly higher amounts of chlorophyll *a* were recorded in ponds fertilized with chicken manure indicating that there was a higher level of phytoplankton production. This is consistent with the work reported by Diana *et al.* (1988) with similar inputs of chicken manure (500 kg/ha/wk). Although nitrogen input was different in the manures used in the present study

(varying from 6 kg/ha/wk for pig manure to 8.04 kg/ha/wk for cattle manure) the influence on natural production was higher in chicken manure. This may be attributed to particle size as fine particles allow faster colonization by bacteria, algae, protozoans and subsequently quicker decomposition and solubilization of key nutrients (Geiger, 1983).

Zooplankton production in ponds varied with type of manure applied. Copepod propagation did not differ significantly in manure treatments and this was similar to that reported by Kamanga and Kaunda (1998). However, the differences from their report were higher numbers of copepods (>100,000/L) than in the present experiment (25,000/L). Cladoceran, especially *Daphnia*, was better in the cattle manure ponds and this was consistent with work reported by Kamanga and Kaunda (1998). *Moina* were more abundant in chicken manure ponds. The rotifers, *Lecanes*, did not differ among the types of fertilizer but were significantly lower in the no-manure treatment. Rotifers were more abundant in the chicken manure ponds. The number of zooplankton was higher in the present experiment than reported by Brummett (2000a) in an organic (napier grass) fertilization regime with *Tilapia rendalli*. In his study, numbers were as low as 1,254/L for rotifers, 76/L for copepods and 29/L for cladocerans. However, Kamanga and Kaunda (1998) reported surprisingly higher numbers of copepods (71,030/L), rotifers (8,530/L) and cladocerans (17,972/L) in no-manure treatment in concrete tanks. These variations may be due to differences in the nutrient levels in the organic manure.

The natural food produced in ponds is high in nutrition and can contribute about 50 to 70% to the growth of tilapia (Schroeder, 1983). Phytoplankton is reported to have protein levels ranging from 12 to 35%, lipids ranging from 7.2 to 23% and carbohydrates

ranging from 8.2 to 8.7% on a dry weight basis. Phytoplankton is considered to also be high in ascorbic acid (Coutteau, 1996). In a related study, Yi *et al.* (2002) reported high levels of ash (51-52%) in some specific phytoplankton species in ponds. The proximate analysis in the present experiment revealed that the protein levels of zooplankton in chicken manure treatment were significantly higher than those from the cattle, pig and no-manure treatments. However, the protein levels of zooplankton in cattle and pig treatments were not significantly different. The trend was the same with gross energy. Ash content significantly differed in all treatments, chicken manure having the highest amount, and with the lowest in no-manure treatment. This was consistent with other studies (Watanabe, 1988; Delbare *et al.*, 1996).

The stomach contents of the fish reflected the food items found in the pond waters. The stomach contents of fish in this experiment included detritus, higher plants, zooplankton, phytoplankton and insects. Brummett (2000a) found the same categories of stomach contents in *T. rendalli* but no insects were noted in his work and plankton was absent in fish ranging from 21-40 g, which was not the case in the present experiment. Fish cultured in my no-manure treatment consumed significantly higher amounts of detritus followed by those in the cattle and pig manure. Fish in the ranges of 20-35 g in this experiment consumed high amounts of detritus and was similar to that reported by Brummett (2000a) for similar organic fertilization regimes. Fish in the chicken manure ponds preferred higher plants, phytoplankton and zooplankton and reduced their intake of detritus. Insects were found the least in stomachs of the fish although those fish in the no-manure treatment had significantly higher amounts of insects in their stomachs. *T.*

rendalli are believed to shift feeding habits as they grow. They change from carnivorous when young (7-33 mm) and consume lots of zooplankton, aquatic insects and detritus, which makes up about 26% of their stomach contents in the wild (Meschiatti and Arcifa, 2002). They become more herbivorous as they grow. Brummett (2000a) noted the shift in his experiment with *T. rendalli* in fertilized ponds.

Detritus was one of the important stomach contents encountered during the analysis and seems to form an important part of *T. rendalli* nutrition. The nutritional quality of detritus from various environments (tropical and temperate) is variable in terms of protein level, which ranges from 2.9 to 24.2% with good amino acid profiles (Bowen, 1987). Sometimes detritus is considered largely unvaluable to an animal's digestive system, but together with detritus, microbes are efficiently digested and provide a rich nutrient diet (Bowen, 1987). It is on the sediment surface, where anaerobic processes are active, that detritus is important as food for animals (Moriarty, 1997).

Organic matter loading due to fertilization was significantly higher in ponds fertilized with cattle manure but they did not differ significantly with chicken and pig manure treatments. The levels of loading were comparable to those reported by Boyd (1990) and Brummett (2000a) in similar fertilization experiments in earthen ponds. This loading was at acceptable levels for organic fertilization. Pond soils tend to acquire greater organic matter concentration than surface soils and loading may increase with organic fertilization (Boyd, 1995). The organic matter acts as substrate for the heterotrophic production of microorganisms and protozoans in microbial food webs that can be utilized by fish to obtain the much needed nutrition through natural crops of algae,

bacteria and other microorganisms in organically fertilized ponds (Geiger, 1983; Boyd, 1995; Moriarty, 1997). My results indicated that concrete ponds function similarly to earthen ponds when you simulate them by adding a layer of soil. They propagate natural production including periphyton (Keshavanath *et al.*, 2002) which spread even in the walls of the concrete ponds.

Water quality in this experiment varied with the type of organic manure applied but did not affect the well-being of the fish. Temperatures were low during this period as it was in the cool season in Malawi. However, temperatures did not differ significantly among treatments. The drop in temperature to the minimum levels of 13.6° C might have slowed the growth rates of the fish, which in turn reduced the yield. Although the temperatures were within the normal range for *T. rendalli* (13.5 - 36° C) according to Philippart and Ruwet (1982), the average temperatures fell below suitable ranges of 20 and 30° C (Maruyama, 1983; Boyd and Tucker, 1998). Growth must have been affected because temperatures dropped three weeks after commencement of the experiment. This trend was similar to what was reported by Rezk *et al.* (2002) where they compared growth of Egyptian tilapias in declining water temperatures. They found that the percent increase at temperatures $\geq 25^{\circ}\text{C}$ ranged from 93% in *O. aureus* to 33% in *T. zilli*; $\leq 20^{\circ}\text{C}$ ranged from 43% in Abbasa population of *O. niloticus* to -3% in *T. zilli*. The *T. rendalli* in the present experiment showed some tolerance to low temperatures and a similar response was noted in another study using *T. rendalli*, which were more cold tolerant than *O. shiranus* (Ohashi *et al.*, 1999).

The other water parameters in the present experiment were within acceptable ranges for tilapia cultured in fertilized ponds (Janassen, 1990; Buttner *et al.*, 1993; Popma and Masser, 1999; Prinsloo *et al.*, 1999; Brummett, 2000a; Bowman, 2002; Chaula *et al.*, 2002). However, the fertilized ponds experienced lower dissolved oxygen (<1.0 mg/l) in the morning than the unfertilized ponds (3.4 mg/l) but improved during the day. The alkalinity levels are generally low in the soils of Malawi despite liming. Jamu (1996) reported low alkalinity levels, which were below 20 mg/l despite liming and indicated that the wide range of lime requirements could reflect similar situations elsewhere in the country. Initially the soil (clay-loam) in the pond was analysed for pH (Boyd, 1979) to determine whether it was necessary to lime during my experiment. It was discovered that there was no need for liming as the pH was within neutral levels (7.1). Alkalinity levels were above 20 mg/l across treatments. Alkalinities at or above 20 mg/L trap CO₂ and increase the concentrations available for photosynthesis (Wurts and Durborow, 1992). Lower than expected levels of alkalinity have detrimental effects in that bio-converter bacteria are destroyed, photosynthesis is hampered and the fish die of ammonia poisoning compounded with pH shock (Meck, 1996).

The overall conditions were also conducive for growth and propagation of zooplankton, such as rotifers, that perform well between 15- 31° C, as well as with dissolved oxygen as low as 1 mg/L (Dert, 1996). Cladocerans such as *Daphnia* and *Moina* grow and propagate well in temperatures ranging from 10 to 25° C, pH of 7-8, at least 1 mg/L of dissolved oxygen and 0.2 mg/L of ammonia (Delbare and Dert, 1996). The dissolved oxygen in my experiment ranged from 0.3-19.9 mg/l. These favorable

environmental conditions also supported high numbers of zooplankton and a demonstration of plentiful phytoplankton and detritus in the ponds (Boyd, 1990; Boyd and Daniels, 1993).

2.5 Conclusion

Fish in chicken manure ponds performed significantly better in this experiment. Chicken manure was able to produce natural food to sustain the high growth rates in *T. rendalli*, increasing yield. Cattle and pig manure did not differ in many aspects throughout the experiment. There were variations in the way the species of zooplankton were propagated with the type of organic manure. Cladocerans (*Daphnia*) were produced at high rates in cattle manure treatment and moina were produced highly in chicken manure treatment. Rotifers were produced at a high rate in chicken manure ponds. However, production of copepods and rotifers did not differ significantly with the type of organic manure used.

Phytoplankton and chlorophyll *a* were significantly higher in chicken manure ponds. The least activity in terms of fish growth and natural food production was in the no-manure treatment. The analysis of stomach contents of the fish and proximate analysis of fish and zooplankton body composition confirmed that more food was available in the fertilized than in unfertilized ponds. Water quality parameters were within range for the growth of *T. rendalli* except for temperature, which was lower than expected for optimal growth. However, survival was high (>90%) showing that the environment had little effect on the fish's survival during this cool season of Malawi.

Chapter 3

EFFECT OF SINGLE INGREDIENT SUPPLEMENTAL FEED ON GROWTH, FEED UTILIZATION, PLANKTON ABUNDANCE AND SURVIVAL OF *TILAPIA RENDALLI* IN CONCRETE PONDS

3.1 Introduction

Some farmers in Malawi practise semi-intensive systems where fertilization and supplementary feeding are used. Supplemental feeds are intended only to help support the natural food available in the ponds, providing nutrients that might limit growth of the fish (Fitzsimmons *et al.*, 1999). Supplements do not contain a full complement of vitamins or minerals, but are used to fortify the natural available diet with extra protein, carbohydrate or lipids (Lovell, 1980; Tacon, 1988; FAO, 1997b; Craig and Helfrich, 2002; Suresh, 2002).

Supplementary feeds usually consist of low cost agricultural/animal by-products, which are much less expensive than complete diets and usually high in carbohydrates. Some supplemental diets serve a dual purpose of fertilizing the pond as well as increasing productivity (Swift, 1993; Fitzsimmons, 1997). Maize bran, rice bran and sorghum have been used in ponds as sole ingredients in several studies (Wohlfarth and Hulata, 1987; Msiska, 1988; Kadongola, 1991; GOM-Min. of Agric., 1993-1994; Chikafumbwa, 1996a; Fitzsimmons *et al.*, 1999). In a study conducted in Israel, Wohlfarth and Hulata (1987) found that yields from intensive manuring, reinforced by feed supplements, were higher than those from manure alone. Lowest yields came from sorghum without manure. They also found that with sorghum supplement, cattle manure appeared to result in higher yield than poultry manure. It appears that different supplements do well with different types of organic fertilization but their combinations are not well known. Recently, Veverica *et al.* (2000) attempted to use rice bran and combinations of organic fertilizers

in ponds stocked with juvenile *Oreochromis shiranus* and *Clarias gariepinus*, in a polyculture system. The net yields ranged from 1.1 to 2.1 tonnes /ha.

Some Malawian fish farmers apply manure and supplements to their fish, but it is rare to use combinations because procurement of these basic pond inputs may be difficult from off farm sources (ICLARM-GTZ, 1991), although might be necessary in such systems to increase yield (Edwards, 1999). These supplements provide additional quantities of nutrients that are needed when the productivity of the water body cannot promote the desired fish growth (Fitzsimmons *et al.*, 1999). Nutrition and feeding of finfish and crustaceans in semi-intensive pond farming systems are complex and poorly understood, and little or no information exists on the dietary requirements of the cultured species (Shroeder, 1983; Hasan, 2001). Although well-fertilized ponds normally have enough high protein natural food, there are difficulties in quantifying the contribution of naturally available food organisms to the overall nutritional budgets of fish raised in ponds (Tacon, 1993). Therefore there is a need to develop a basic understanding of nutrient dynamics, especially the role of fertilization, feeding and natural productivity (Hasan, 2001) to increase the production of fish in semi-intensive systems. Many locally available ingredients are not well utilized as single ingredient supplements in pond aquaculture in Malawi (World Bank, 1988).

Single supplements that have been used in Malawi by farmers include maize bran and rice bran (Kadongola, 1991; Jamu and Costa-Pierce, 1995). There are also many other sources of protein such as soybean, cotton seed cake, sunflower cake, sunflower bran, pigeon peas and pigeon pea bran that can be used as single supplements in ponds.

This experiment compared the use of three different single ingredients in fertilized ponds, with the aim of evaluating the effect of applying single ingredient supplementary feed on the growth, plankton abundance and survival of *T. rendalli* raised in ponds fertilized with poultry manure.

3.2 Materials and methods

3.2.1 Experimental facilities and set-up

The experiment was carried out from September to December 2002 in ponds at Bunda College of Agriculture fish farm, a facility of the Department of Aquaculture and Fisheries Science, University of Malawi. It was conducted. During the supplementary feed trial, ponds were fertilized with chicken manure at the rate of 500 kg/ha/wk. Pond preparation was as described in section 2.2.1 (Chapter 2). Supplementary treatments of soybean, maize bran, rice bran and chicken manure only as a control were assigned to the ponds at random in a completely randomised design (CRD) with three replicates each.

3.2.2 Feeds and feeding

The ingredients used in this experiment were maize bran, soybean and rice bran. Maize bran and soybean were obtained from villages around Bunda College. Rice bran was obtained from the Govala market in the Zomba area, about 250 km from the experimental station.

Proximate analysis of feed ingredients was performed before the commencement of the experiment. The ingredients were ground to pass through a 0.2 mm sieve before they were assayed for proximate analysis using standard methods (AOAC, 1990) as

outlined below. The proximate analysis included dry matter, crude protein, crude fat, crude fibre, ash and gross energy. Other components analysed were available lysine (spectrophotometry), trypsin inhibitor (Folin C method-calorimetry) and minerals (spectrophotometry). Nitrogen free extract and organic matter were calculated by difference.

3.2.2.1 Dry matter determination

Determination of dry matter of the sample was achieved by drying a pre-weighed sample in an oven at 105° C until a constant weight was achieved.

3.2.2.2 Crude protein determination

The Kjeldahl procedure was used to determine the crude protein content of the samples. The samples were digested in concentrated sulfuric acid, distilled and then titrated with standard 0.05N sodium hydroxide solution to quantify the nitrogen content which was then converted to protein using the conversion factor of 6.25. This conversion factor was used because, on average, protein contains 16% nitrogen (AOAC, 1990) hence the nitrogen content was multiplied by 6.25 ($100/16$) to calculate protein content of the sample.

3.2.2.3 Crude fat determination

The Soxhlet apparatus method was used in the fat extraction from the samples using hot petroleum ether for 14-16 hours. After draining the ether, the flasks containing

the fat were dried in an oven for about 8 hours at 85-90° C. The increase in the weight of the flask was due to crude fat and the crude fat content was calculated by dividing the weight of the ether extract by the sample dry weight.

3.2.2.4 Crude fibre determination

Usually crude fibre is determined by utilizing the residues from the crude fat determination. The sample was boiled in weak acid, 0.1 M HCl, followed by weak base, 0.313M sodium hydroxide. The samples were then put in the muffle furnace at 550° C for 2 hours and cooled. The loss in weight, which represented the fibre in the original sample, was calculated by subtracting the weight of ash from the initial weight of the sample.

3.2.2.5 Ash determination

The ash content was determined by putting the samples in a muffle furnace at 550° C for 16 hours. Ash was calculated by dividing the weight of ash by the weight of the sample.

3.2.2.6 Nitrogen free extract determination

This category of compounds includes the simple sugars, compound sugars and soluble polysaccharides such as starch. The nitrogen free extract (NFE) was determined by calculating the amount remaining after the ingredients were subtracted. Hence, NFE was determined by taking $100\% - (\% \text{ crude protein} + \% \text{ crude fibre} + \% \text{ fat} + \% \text{ ash})$.

3.2.2.7 Available lysine determination

Available lysine was determined using the procedure outlined by Carpenter (1960). The samples were refluxed for 20 hours in 6N HCl, diluted, titrated and read for absorbance. Carpenter's available lysine method uses a dye called Fluoro-2,4-dinitrobenzine which reacts with the end nitrogen of lysine often called the E-amino group of lysine, and produces a coloured compound called DNP-lysine whose absorbance is analysed on a spectrophotometer. The available lysine was then quantified by using the standard curve. The % available lysine was calculated by dividing the weight of available lysine by the weight of the sample multiplied by 100.

3.2.2.8 Trypsin inhibitor determination

Trypsin inhibitor was determined in full fat soybeans, which were oven roasted at 245° C for 45 minutes. The Folin C method was used for determination of trypsin inhibitor activity. The extracts from the sample were diluted and assayed for trypsin activity. Casein and 5% CTA were added and the concentration of peptides was determined by adding 1.0 M NaOH and folinciocalteau reagent. Absorbance was read on the colorimeter. Calculations of the percent activity were done by dividing the absorbance change for the treatment by the absorbance change for the standard and multiplying by 100. The percentage inhibition for the sample was calculated by subtracting the percentage activity from 100.

3.2.2.9 Gross energy determination

Gross energy was determined by igniting the sample in a bomb calorimeter. The gross energy of the food is measured by combustion, usually in an atmosphere of compressed oxygen. Under these conditions carbon and hydrogen are fully oxidised to carbon dioxide, water and other gases. The heat released is then measured. In this study, a Nenkem type adiabatic bomb calorimeter was used.

3.2.2.10 Organic matter determination

Organic matter is taken as the material, which normally burns in the presence of oxygen, while ash or mineral matter remains. After the ash content was analysed, the organic matter was calculated as the difference (El-Sayed, 2003). Therefore, the % organic matter on a dry matter basis was calculated as % dry matter - % ash.

3.2.2.11 Mineral analysis

Mineral analysis was done by ashing the samples in the muffle furnace at 550° C for 16 hours. The samples were then put on a hot plate with sand for acid digestion in concentrated 50% HCl followed by dilute acid 1N HCl. The digestion process allowed the acid to dry half way in the crucibles and then was refilled to make sure there was no loss of some minerals. The liquid ash was quantitatively transferred for filtering with deionized water in a volumetric flask. The filtrate was further diluted for reading on Atomic Absorption Spectrophotometer (AAS). Calculations were made basing on the

readings on the AAS. The minerals analysed were phosphorus, calcium and potassium (Table 3.1).

Table 3.1 Proximate analysis of the ingredients used as supplements fed to *Tilapia rendalli* in ponds (dry weight)(Mean \pm SD).

Component Analysed (%)	Soybean	Maize bran	Rice bran
Dry Matter (DM) ¹	93.2 \pm 1.9	91.7 \pm 0.7	91.5 \pm 0.4
Crude Protein (CP)	42.2 \pm 0.7	13.1 \pm 0.1	12.5 \pm 0.5
Crude Fibre (CF)	6.0 \pm 0.1	3.7 \pm 0.1	11.3 \pm 0.1
Crude fat	20.8 \pm 0.7	10.4 \pm 0.3	7.1 \pm 0.3
Ash	4.45 \pm 0.17	3.26 \pm 0.27	10.6 \pm 0.5
Phosphorus	0.56 \pm 0.02	0.52 \pm 0.04	0.22 \pm 0.02
Calcium	0.09 \pm 0.03	0.04 \pm 0.01	0.02 \pm 0.00
Potassium	0.49 \pm 0.03	0.36 \pm 0.01	0.11 \pm 0.01
NFE	26.6 \pm 0.7	69.6 \pm 0.2	58.6 \pm 0.5
Organic matter	88.8 \pm 1.7	88.4 \pm 0.5	80.9 \pm 0.1
Available Lysine (%)	4.4 \pm 0.3	-	-
Trypsin Inhibition (%)	44.1 \pm 0.6	-	-
Gross Energy (kJ g ⁻¹)	20.8 \pm 0.1	18.5 \pm 0.1	16.3 \pm 0.1

- Not analysed; ¹ % wet weight basis; NFE- Nitrogen free extract.

3.2.3 Pond fertilization regime

Chicken manure was collected from a livestock farm within Bunda College of Agriculture, transported while dry and kept in sacks in the shade. The ponds were fertilized with poultry manure at the rate of 500 kg/ha/wk two weeks before the fish were stocked into ponds and continued throughout the experimental period. The manure was applied at this rate based on the previous recommended rates for chicken manure and maintained as in the previous fertilization experiment (Chapter 2). Manure was applied by hand broadcasting, except on windy days where the manure was mixed in a bucket with water and added to ponds.

3.2.4 Biochemical analysis of organic manure used

The manure was analysed for dry matter, nitrogen, phosphorus and potassium during this experiment as described in section 2.2.3 (Chapter 2). The assumption was that there would be differences since the manure was collected for the second time from the same source as in the fertilization experiment (Table 3.2).

3.2.5 Fish stocking and sampling

The initial weight and total length of the fish used during this experiment averaged 5.73 ± 0.66 g and 6.72 ± 0.38 cm, respectively. The acclimation, stocking and sampling procedures were as described in section 2.2.4 (Chapter 2).

3.2.6 Fish growth performance

Total body weight (g), standard and total lengths (cm) of fish were taken. Mortality was recorded to monitor survival. Fish growth rate performance was calculated as weight gain, percentage (%) increase in average weight, specific growth rate (%/ day), gross yield of fish (kg/ha), net yield of fish (kg/ha), survival rate (%) and Fulton's condition factor. These were calculated using the formulae as outlined in section 2.2.5 (Chapter 2).

Table 3.2 Proximate composition of organic manure applied in experimental ponds stocked with *Tilapia rendalli* (Mean \pm SD).

Proximate component (%)	Organic manure source
	Chicken
Dry matter (% wet weight)	90.18 \pm 0.62
Nitrogen (% dry weight)	1.23 \pm 0.05
Phosphorus (%dry weight)	1.40 \pm 0.03
Potassium (% dry weight)	0.61 \pm 0.01

3.2.7 Feed preparation and feeding

The ingredients were milled so they would pass through a 0.2 mm sieve and then mixed with water (about 500 ml per kg of feed) to form a dough that could be pelleted. A hand pelleter (mincer) was used to produce pellets of about 2 mm (Fig. 3.1). These were airdried and stored in a freezer. The fish were fed crumbled pellets at the rate of 3%

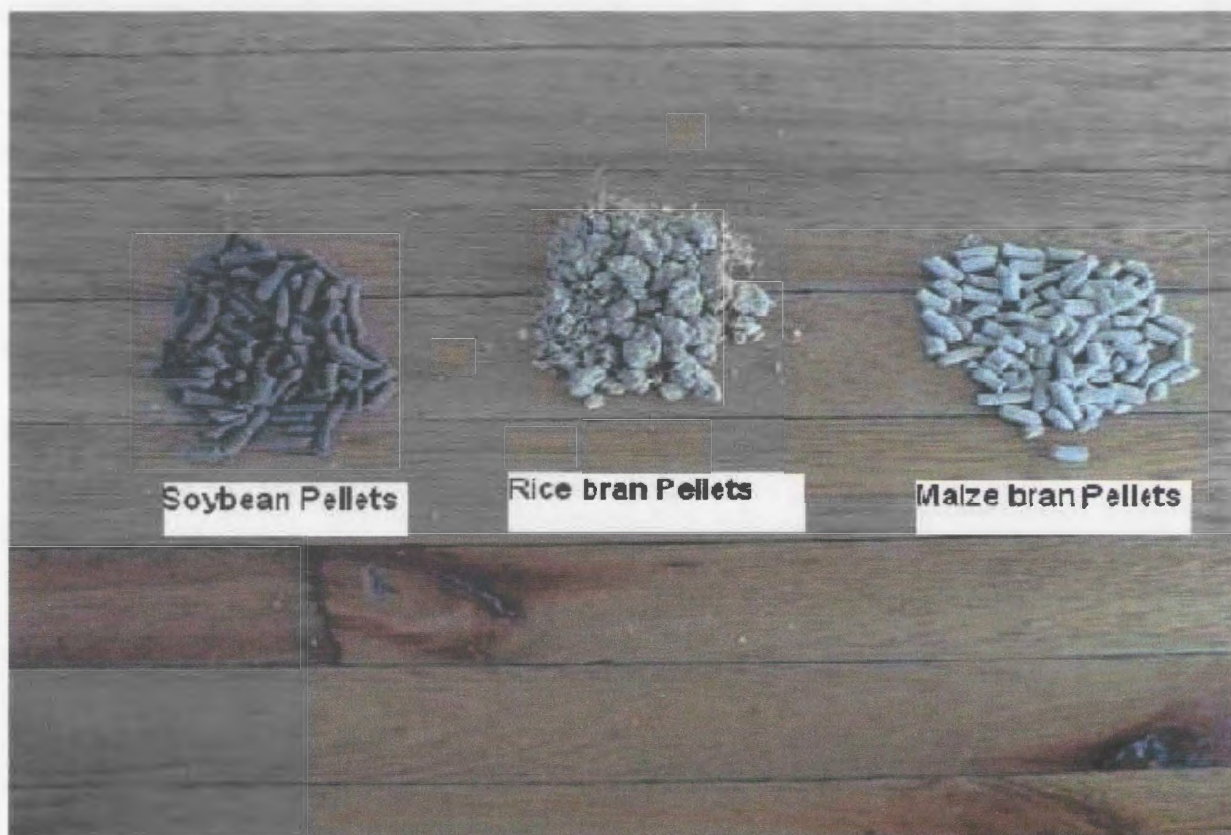


Fig .3.1. Airdried pellets of different types of supplementary feeds provided to *Tilapia rendalli* in ponds.

body weight by broadcasting by hand every day 1.5% at 08:00 hr and the other 1.5% at 14:00 hr except the day preceding sampling day when the fish were not fed. The amount of supplementary feed provided was adjusted after weighing the fish at each sampling.

3.2.8 Apparent feed utilization

Apparent feed utilization (Watanabe, 1988) was calculated as feed conversion ratios (FCR) = dry weight of feed (g)/ weight gain (g) and feed conversion efficiencies (FCE) = weight gain (g)/ feed consumption (g) x 100.

3.2.9 Plankton monitoring and enumeration

Plankton enumeration was conducted as in the fertilization experiment section 2.2.6 (Chapter 2) where zooplankton (individuals/L) was enumerated and samples of phytoplankton (chlorophyll *a*, µg/L) were analysed once a week using appropriate methods (APHA, 1985; Gall *et al.*, 2000; Brummett, 2000).

3.2.10 Chemical composition of fish and zooplankton

Proximate analysis was done on the fish before stocking and at the end of the experiment to evaluate if there was an effect due to treatment. The zooplankton was harvested and subjected to proximate analysis as well. Both fish and zooplankton were assayed for moisture, crude protein, crude fat, ash and gross energy using standard methods (AOAC, 1990). The procedure used was as described in section 2.2.7 (Chapter 2).

3.2.11 Pond sediments analysis

Pond soil was collected once a month during this experiment to assess the organic matter loading. The analysis procedure was as described in section 2.2.9 (Chapter 2).

3.2.12 Stomach contents analysis

Analysis of stomach contents was performed as described in 2.2.8 (Chapter 2).

3.2.13 Water quality monitoring

The water quality parameters monitored and methods used were as described in section 2.2.10 (Chapter 2).

3.2.14 Data collection and statistical analysis

The data collection procedure was as described in Chapter 2. The data were first checked for assumptions for analysis of variance. The analysis was performed to test the effect of type of supplementary feed. All statistical analyses were carried out using SPSS program, 10.0 (SPSS Inc., 1999) and presentation of the data was as described in section 2.2.11 (Chapter 2).

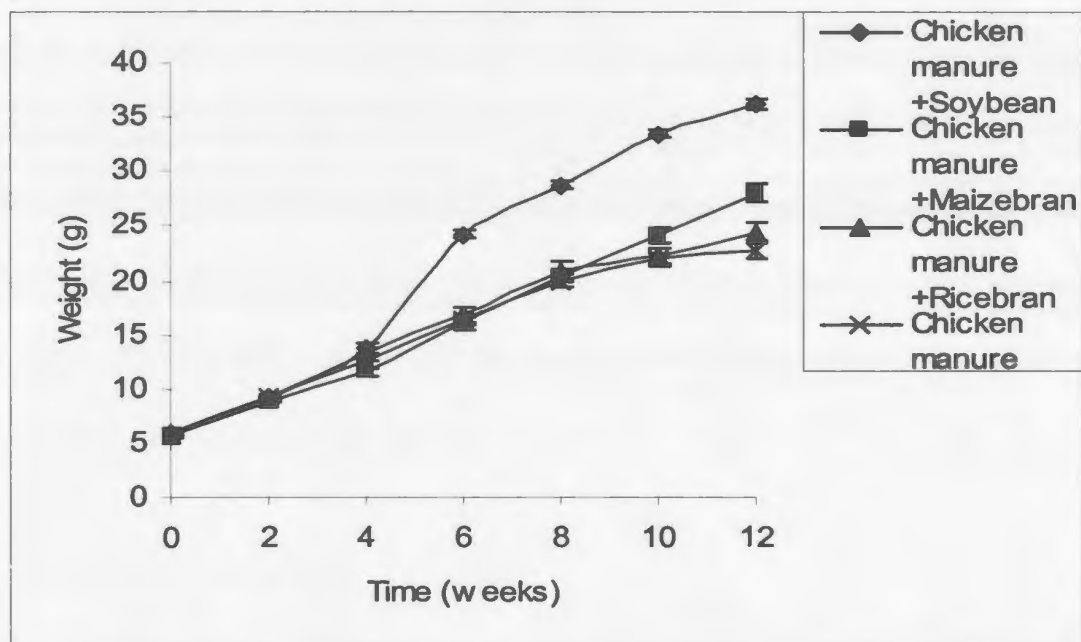
3.3 Results

3.3.1 Growth of fish

Data met assumptions of ANOVA. Significant ($P < 0.05$) differences in the growth of fish began to appear in the fourth week (Fig. 3.2). The initial weight of the fish did not differ significantly among treatments. Fish weights in the soybean and rice bran treatments were significantly higher than for the fish in the maize bran and chicken manure treatments, which did not differ significantly from each other in the second sampling (Appendix 3.1). Fish weight in the rice bran and chicken manure ponds was significantly lower than the other treatments, but fish in ponds fed soybean grew faster and differed significantly from the rest of the treatments throughout the experimental period. The fish grew from an average weight of 5.73 g to 36.17 g in soybean, 27.95 g in maize bran, 24.43 g in rice bran and 22.83 g in the chicken manure treatments (Fig. 3.2; Appendix 3.1). The growth curves also indicate that the fish increased significantly more in both weight and length in the soybean treatment after four weeks in the experiment (Fig. 3.2).

The total weight gain of fish differed significantly among treatments. Fish in the soybean treatment had significantly higher weight gain (30.3 g), followed by the fish fed the maize bran (22.22 g), rice bran (18.65 g) and chicken manure (17.26 g) treatment, but the rice bran and chicken manure treatments fish did not differ significantly from each other (Table 3.3). Weight gain per day followed the same trend of total weight gains and ranged from 0.20 g/day for chicken manure to 0.36 g/day for fish in the soybean treatment.

a)



b)

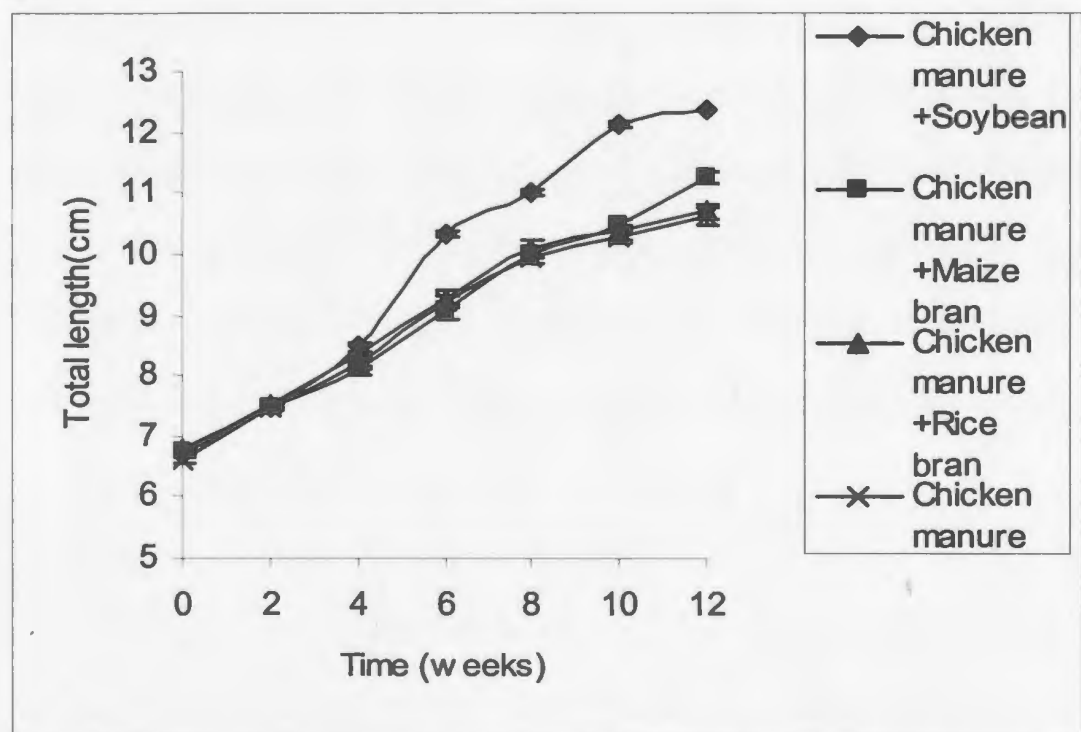


Fig. 3.2. Mean (\pm SE) of weight (a) and total length (b) of *Tilapia rendalli* grown in ponds fertilized with chicken manure and fed different supplementary feeds (N=60).

The percent increase in weight of fish was significantly ($P < 0.05$) higher in the soybean treatment (523%) followed by the maize bran (394%), rice bran (329%) and chicken manure treatment (319%). Specific growth rate followed the trend of percent increase, which was significantly higher for fish fed the soybean treatment (2.17 % /day) followed by fish fed the maize bran treatment (1.87 %/day). Growth rates of fish fed rice bran (1.67 %/day) and chicken manure (1.64 %/day) were lower and did not differ significantly from each other (Table 3.3).

3.3.2 Feed utilization

Apparent feed utilization among treatments differed significantly ($P < 0.05$) with the supplementary feed used. Fish fed rice bran had significantly higher feed conversion ratio (2.17) than soybean (1.5) and maize bran (1.64), which did not differ significantly. The feed conversion efficiency followed the same trend of feed conversion ratios. Fish fed rice bran had significantly lower efficiency (54.5%) than the fish fed soybean (67.5%) and maize bran (65.8%). The fish in ponds supplied with soybeans utilized the feed better than the fish in maize bran and rice bran treatments (Table 3.3).

3.3.3 Yield, survival and condition of fish

There were significant differences ($P < 0.05$) among treatments in gross yield (kg/ha), net yield (kg/ha) and extrapolated net annual yields (kg/ha/yr). The extrapolated net yields followed the same trend of gross and net yields (Table 3.4). Net annual yields were significantly higher in fish from the soybean treatment (2,279 kg/ha/yr) followed by fish yields from maize bran treatment (1,648 kg/ha/yr), rice bran (1,361 kg/ha/yr)

Table 3.3 Initial weight, final weight, weight gain, weight gain per day, weight increase, specific growth rate (SGR), feed conversion ratio (FCR) and feed conversion efficiency (FCE) of *Tilapia rendalli* in treatment ponds fertilized with chicken manure and fed different supplementary feeds (Mean \pm SE).

Parameter	Treatment			
	Chicken manure + Soybeans	Chicken manure + Maize bran	Chicken manure +Rice bran	Chicken manure
Initial wt (g)	5.87 \pm 0.08	5.73 \pm 0.08	5.78 \pm 0.09	5.56 \pm 0.09
Final weight (g)	36.17 \pm 0.43 ^c	27.96 \pm 0.83 ^b	24.43 \pm 1.00 ^a	22.83 \pm 0.79 ^a
Total wt gain (g)	30.30 \pm 0.43 ^c	22.22 \pm 0.82 ^b	18.65 \pm 1.01 ^a	17.26 \pm 0.81 ^a
Weight gain/day	0.36 \pm 0.01 ^c	0.24 \pm 0.01 ^b	0.22 \pm 0.01 ^a	0.20 \pm 0.01 ^a
Weight increase (%)	523 \pm 11 ^c	394 \pm 16 ^b	329 \pm 19 ^a	319 \pm 17 ^a
SGR (%/day)	2.17 \pm 0.02 ^c	1.87 \pm 0.04 ^b	1.67 \pm 0.05 ^a	1.64 \pm 0.05 ^a
FCR	1.50 \pm 0.02 ^a	1.64 \pm 0.06 ^a	2.17 \pm 0.12 ^b	-
FCE	67.5 \pm 1.0 ^b	65.8 \pm 2.4 ^b	54.5 \pm 3.0 ^a	-

ANOVA, N=240, Initial wt: $F_{3, 236} = 2.249$, $P = 0.083$; Final wt: $F_{3, 236} = 56.986$, $P < 0.001$; weight gain: $F_{3, 236} = 53.734$, $P < 0.001$; Weight Incr: $F_{3, 236} = 33.989$, $P < 0.001$; SGR: $F_{3, 236} = 34.786$, $P < 0.001$; N=180, Log₁₀ FCR: $F_{3, 177} = 16.842$, $P < 0.001$; FCE: $F_{3, 177} = 9.633$, $P < 0.001$.

Note: Values with different superscripts in a row are significantly different (ANOVA, $P < 0.05$); - not calculated.

and the chicken manure treatment (1,290 kg/ha/yr). However, the net annual yield did not differ significantly between fish from the rice bran and chicken manure treatments.

Fish survival rate was over 90% among the treatments. The highest survival was recorded in both chicken manure and soybean (94.2%), and the lowest in both maize bran and rice bran treatments (93.3%). However, fish survival rates did not differ significantly ($P > 0.05$) among treatments (Table 3.4).

There were no significant differences in the initial and final condition factors of the fish among treatments, although the final values were lower than initial condition factors in the soybean and chicken manure treatments compared to the condition factors of fish in maize and rice bran, which increased (Table 3.4).

3.3.4 Whole body composition of fish

There were significant ($P < 0.05$) differences among the fish in the treatments in their whole body composition of moisture, ash, fat, protein and gross energy. The final composition was compared to the samples from the beginning of the experiment. There was an increase in moisture in fish fed the soybean treatment which was significantly lower than the moisture of fish in the maize bran (68.08%), rice bran (68.08%) and chicken manure (67.9%) treatment, which did not differ significantly in their moisture contents (Table 3.5).

There was a decrease in ash content among the fish in all the treatments from the initial composition. The highest ash content was found in the initial sample of the fish. The fish from the rice bran and chicken manure treatments were lower in ash content than

Table 3.4 Gross yield, net yield, net annual yield, survival, initial and final condition of *Tilapia rendalli* in treatment ponds fertilized with chicken manure and fed different supplementary feeds (Mean \pm SE).

Parameter	Treatment			
	Chicken manure +Soybean	Chicken manure +Maize bran	Chicken manure +Rice bran	Chicken manure
Gross yield (kg/ha)	687 \pm 8 ^c	527 \pm 16 ^b	456 \pm 19 ^a	434 \pm 15 ^a
Net yield (kg/ha)	570 \pm 8 ^c	412 \pm 16 ^b	340 \pm 19 ^a	322 \pm 15 ^a
Net ann.yd (kg/ha/yr)	2,279 \pm 34 ^c	1648 \pm 62 ^b	1361 \pm 76 ^a	1290 \pm 62 ^a
Survival (%)	94.2 \pm 0.8	93.3 \pm 3.6	93.3 \pm 0.0	94.2 \pm 2.2
Initial condition	1.94 \pm 0.02 ^b	1.87 \pm 0.02 ^{ab}	1.83 \pm 0.02 ^a	1.96 \pm 0.05 ^b
Final condition	1.92 \pm 0.02	1.94 \pm 0.02	1.94 \pm 0.02	1.88 \pm 0.03

ANOVA, N=240, Gross yield: $F_{3, 236} = 59.760$, $P < 0.001$; Net yield: $F_{3, 236} = 56.197$, $P < 0.001$; Net annual yield: $F_{3, 236} = 56.197$, $P < 0.001$; Survival: $F_{3, 8} = 0.099$, $P < 0.958$; Initial condition: $F_{3, 236} = 3.715$, $P = 0.012$; Final condition: $F_{3, 236} = 1.252$, $P = 0.292$;

Note: Values with different superscripts in a row are significantly different (ANOVA, $P < 0.05$)

the fish in rest of the treatments but did not differ significantly from each other (Table 3.5). Fat content increased from the initial composition across treatments. The fat content was significantly higher in fish cultured in the soybean treatment (16.66%) followed by the fish from maize bran (16.01%) treatment. The fat content of the fish from the rice bran (15.31%) and chicken manure (15.21%) treatments did not differ significantly (Table 3.5).

The protein level of the fish decreased from the initial level but the drop in protein did not differ significantly among the treatments (Table 3.5). Gross energy level increased from initial energy content and there were significant ($P < 0.05$) differences among the treatments with fish cultured in the soybean treatment having the highest energy level (31.5 kJ/g), followed by maize bran (26.8 kJ/g), rice bran (26.1 kJ/g) and lastly those cultured in chicken manure alone (24.5 kJ/g) (Table 3.5).

3.3.5 Effect on plankton abundance

There were variations in plankton abundance in the pond cultured with *T. rendalli* in both zooplankton (number/L) and chlorophyll *a* ($\mu\text{g/L}$). The copepods (*Cyclops*) were significantly ($P < 0.05$) higher in the soybean treatment than the rest of the treatments. However, *Cyclops* numbers were not significantly different among maize bran, rice bran and chicken manure treated ponds. The other genera of copepods, the *Cladidae*, was significantly higher in both soybean (13,898/L) and chicken manure (13,758/L) treated ponds. The numbers of *Cyclops* in maize bran (8,922/L) and rice bran (10,665/L) treated ponds did not differ significantly from each other (Fig. 3.3).

Table 3.5 Whole body composition (moisture, ash, crude fat, crude protein and gross energy) of *Tilapia rendalli* in treatment ponds fertilized with chicken manure and fed different supplementary feeds (Mean \pm SE).

Treatment	Parameter				
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Gross Energy (kJ/g)
Initial	64.46 \pm 0.32 ^a	14.09 \pm 0.08 ^d	9.53 \pm 0.12 ^a	67.60 \pm 0.13 ^b	18.6 \pm 0.28 ^a
Chicken manure					
+Soybean	67.14 \pm 0.14 ^b	10.24 \pm 0.03 ^c	16.66 \pm 0.05 ^d	66.78 \pm 0.07 ^a	31.5 \pm 0.11 ^c
Chicken manure					
+Maize bran	68.08 \pm 0.10 ^c	8.92 \pm 0.05 ^b	16.01 \pm 0.03 ^c	66.76 \pm 0.07 ^a	26.8 \pm 0.06 ^d
Chicken manure					
+Rice bran	68.08 \pm 0.18 ^c	8.67 \pm 0.05 ^a	15.31 \pm 0.04 ^b	66.85 \pm 0.10 ^a	26.1 \pm 0.18 ^c
Chicken manure	67.9 \pm 0.17 ^c	8.58 \pm 0.06 ^a	15.21 \pm 0.05 ^b	66.61 \pm 0.12 ^a	24.5 \pm 0.15 ^b

ANOVA, N=39, Moisture : F_{4,34} =47.329, P<0.001; Ash: F_{4,34} =1094.156, P<0.001; Fat: F_{4,34} =1515.061, P<0.001; Protein: F_{4,34} =5.970, P=0.001; Gross energy : F_{4,34} =695.438, P<0.001;

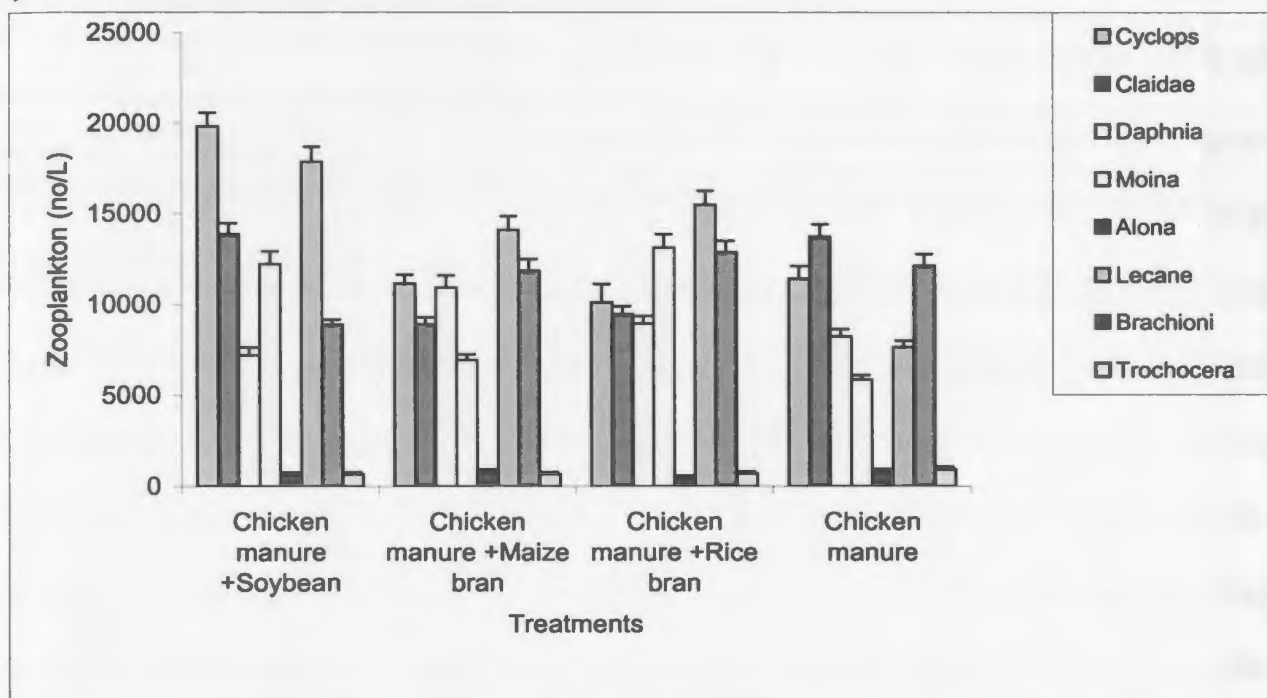
Note: Values with different superscripts in a column are significantly different (ANOVA, P< 0.05)

The cladocerans of the *Daphnia* and *Moina* genera were significantly different in their numbers with treatment but the alona class was not significantly ($P > 0.05$) different among treatments. The *Daphnia* numbers were significantly higher in maize bran (8,958/L) than the rest of the treatments. The *Moina*, however, were high in soybean (12,253/L) and rice bran (13,161/L) but not significantly different from each other (Fig. 3.3).

The rotifers were significantly different in ponds among the treatments. The *Lecanes* were significantly lower in manure treated ponds (7,660/L) and highest in soybean treated ponds (17,176/L). The *Lecane* numbers in maize bran (14,135/L) and rice bran (15,513/L) did not differ significantly from each other. However, the *Brachioni* were significantly lower in soybean treated ponds than maize bran, rice bran and chicken manure treatments, which did not differ significantly (Fig. 3.3). The *Trochocera* did not differ significantly ($P > 0.05$) among the treatments and was the lowest of rotifers with less than 1000/L in all treatments (Fig. 3.3).

Phytoplankton abundance, measured as chlorophyll *a*, was the highest in the soybean treatment (101.9 $\mu\text{g/L}$) followed by chicken manure (89.0 $\mu\text{g/L}$), maize bran (85.9 $\mu\text{g/L}$) and lastly rice bran (82.8 $\mu\text{g/L}$). However, the chlorophyll *a* content did not differ significantly among chicken manure, maize bran and rice bran treatments. Appendix 3.2 provides zooplankton numbers and chlorophyll *a*.

a)



b)

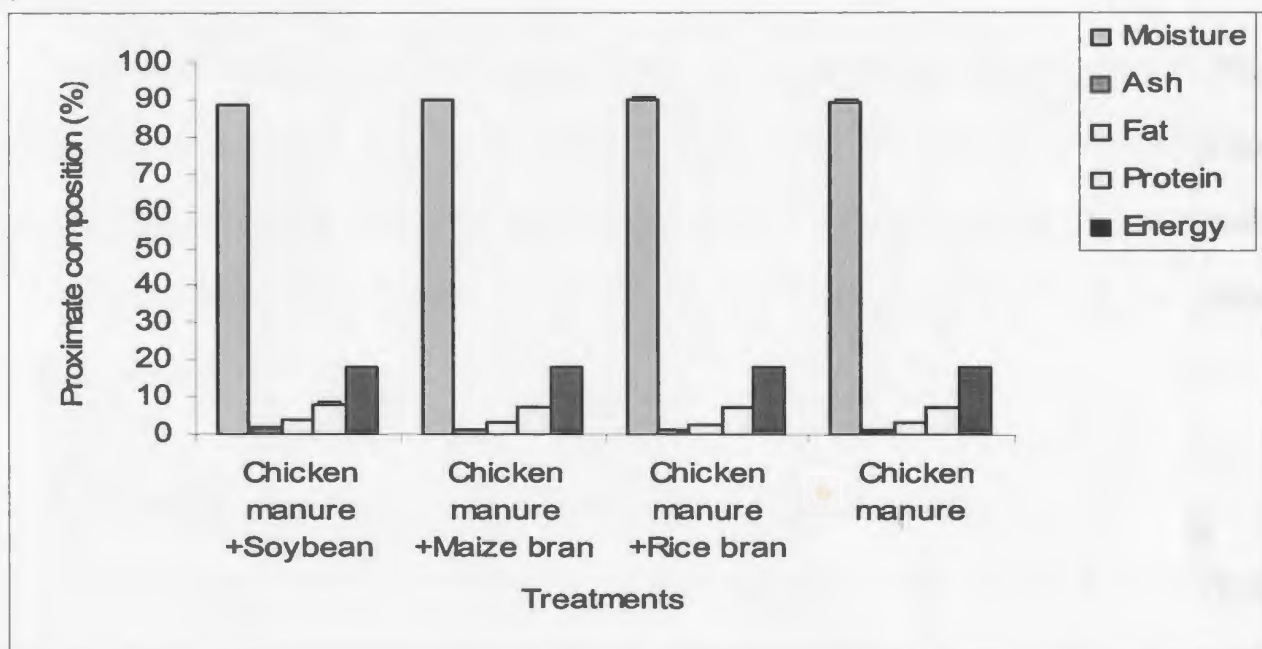


Fig. 3.3. Mean (\pm SE) of population sizes of different classes of zooplankton (a) and their proximate composition (b) in treatment ponds stocked with *Tilapia rendalli* fertilized with chicken manure and provided with different supplementary feeds.

3.3.6 Effect on zooplankton body composition

The analysis of zooplankton body composition indicated significant ($P < 0.05$) differences among treatments for moisture, ash, fat, protein and gross energy content. Zooplankton in the soybean treatment had a significantly lower level of moisture (88.31%) compared to the rest of the treatments, which did not differ significantly from each other (Fig. 3.3). Ash content was significantly ($P < 0.05$) higher in the soybean treatment (1.96%) compared to maize bran (1.46%), rice bran (1.35%) and chicken manure (1.42%). Fat content of zooplankton was significantly higher in soybean (4.06%) ponds followed by maize bran (3.21%), chicken manure and lastly rice bran (2.80%). However, fat content in maize bran and chicken manure treatments did not differ significantly from each other (Fig. 3.3).

Protein content of the zooplankton was significantly higher in soybean (8.33%) than maize bran (7.6%), rice bran (7.34%) and chicken manure (7.28%), which did not differ significantly. The highest amount of energy was found in zooplankton from ponds fed maize bran but the rest of the treatments did not differ significantly from each other (Fig. 3.3)(Appendix 3.3).

3.3.7 Stomach contents of fish and pond organic matter loading

The contents found in stomachs of fish in this experiment ranged from detritus, higher plants, zooplankton, phytoplankton, insects, feed and unidentified material. Detritus was found in large amounts in ponds treated with rice bran and chicken manure, 38.4 and 38.2%, respectively and did not differ significantly from each other (Fig. 3.4). The stomachs of fish cultured with soybean and maize bran supplementary feeds had

significantly lower amounts of detritus, 30.0 and 29.3% respectively, which did not differ significantly. Higher plants were significantly ($P < 0.05$) higher in soybean (22.2%) and maize bran (20.4%) treated ponds, which did not differ significantly from each other. Low amounts of higher plants were found in stomachs of fish cultured with rice bran (13.3%) and chicken manure (11.7%) ponds, but did not differ significantly from each other (Fig. 3.4).

Zooplankton was found in substantial amounts in the stomachs of *T. rendalli*, especially in stomachs of fish cultured with chicken manure (18.4%), followed by those with maize bran (16.3 %). The stomachs of fish in soybean and rice bran treatments had significantly lower amounts of zooplankton and did not differ significantly from each other (Fig. 3.4). Phytoplankton was found in significantly higher amounts in stomachs of fish cultured in maize bran followed by soybean and rice bran treatments, which did not differ significantly between each other. However, phytoplankton was significantly lower in stomachs of fish cultured in chicken manure (Fig. 3.4).

Insects were found in low amounts across the treatments and did not differ significantly. The supplementary feed found in the stomachs of fish in the lowest percentage was 0.13% for soybean and maize bran, and 0.15% in rice bran fed ponds. The others did not differ significantly among the treatments (Fig. 3.4, Appendix 3.4).

The loading of organic matter was significantly higher in rice bran treated ponds than the rest of the treatments, which did not differ significantly from each other. (Appendix 3.4).

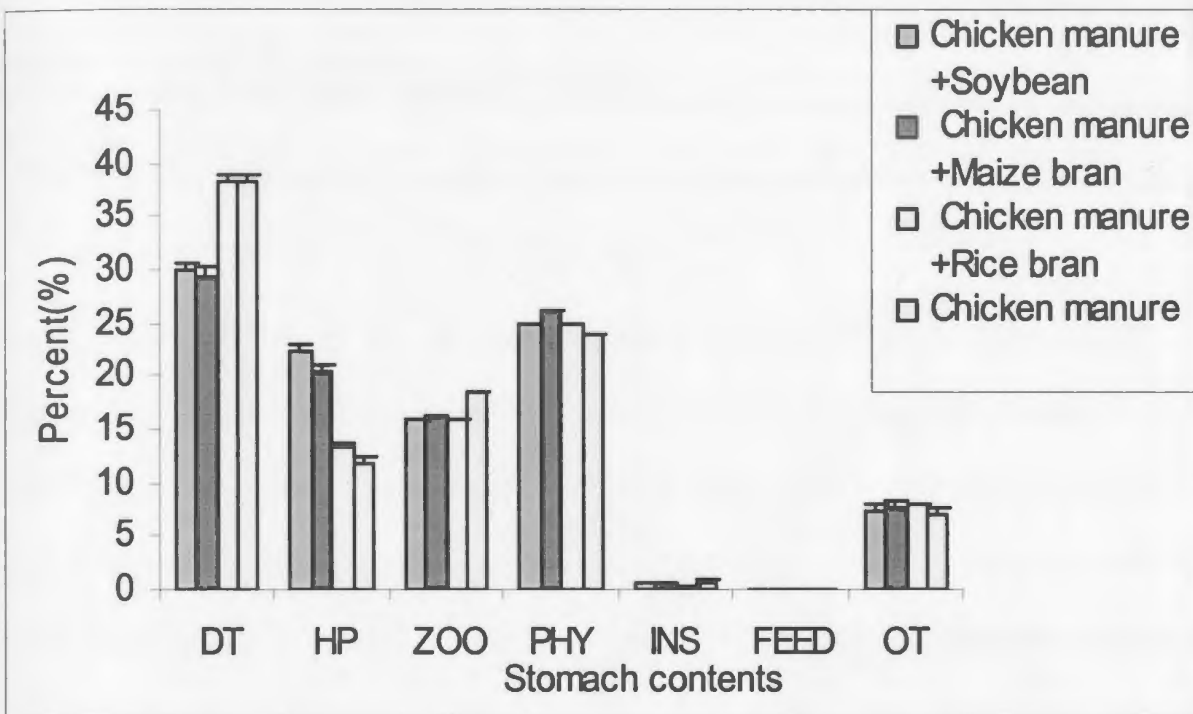


Fig 3.4. Mean (\pm SE) of stomach contents, detritus (DT), higher plants (HP), zooplankton (ZOO), phytoplankton (PHY), feed (FEED) and others (OT) of *Tilapia rendalli* in treatment ponds fertilized with chicken manure and fed different supplementary feeds.

3.3.8 Water quality parameters

Water quality parameters measured during the experimental periods were within the range for growth of *T. rendalli* in ponds. Mean pH (7.84 -7.92), conductance (466-471 $\mu\text{S cm}^{-1}$), mean dissolved oxygen (7.1-7.9 mg/L), mean temperatures (22.8-23° C), salinity (0.01‰), ammonia (0.17 mg/L), nitrite (0.01 mg/L) and secchi disk visibilities (30.6-31.7 cm) did not differ significantly among treatments (Table 3.6, Appendix 3.5 and Appendix 3.6).

However, alkalinity, phosphorus and calcium differed significantly among treatments. Alkalinity was significantly ($P < 0.05$) higher in soybean treated ponds (49.1 mg/L) than maize bran (46.6 mg/L), rice bran (45.4 mg/L) and chicken manure (48.0 mg/L). However, the alkalinity level in maize bran, rice bran and chicken manure did not differ significantly from each other. Phosphorus was significantly higher in soybean and chicken manure (0.19 mg/L). Significantly lower phosphorus levels were recorded in maize bran and rice bran ponds (0.18 mg/L). Calcium was also significantly higher in soybean treated ponds (7.90 mg/L) followed by the ponds applied with maize bran, chicken manure and lastly significantly lower calcium was recorded in rice bran applied ponds (Table 3.6).

Table 3.6 Water quality parameters measured in treatment ponds fertilized with chicken manure and supplied with different supplementary feeds and stocked with *Tilapia rendalli* for 84 days (Mean \pm SE).

Parameter	Treatment			
	Chicken manure +Soybean	Chicken manure +Maize bran	Chicken manure +Rice bran	Chicken manure
pH	7.90 \pm 0.02 ^{ab}	7.84 \pm 0.03 ^a	7.94 \pm 0.03 ^b	7.92 \pm 0.02 ^{ab}
Conduc (μ S cm ⁻¹)	468 \pm 4	471 \pm 4	468 \pm 4	466 \pm 4
Oxygen (mg/L mean)	7.9 \pm 0.6	7.1 \pm 0.1	7.6 \pm 0.2	7.6 \pm 0.1
Oxygen (mg/L range)	0.2 – 19.9	0.1- 16.3	0.2 – 19.9	0.7 – 19.0
Temp ($^{\circ}$ C mean)	23.0 \pm 0.1	22.8 \pm 0.1	23.0 \pm 0.1	22.8 \pm 0.1
Temp ($^{\circ}$ C range)	14.4 - 30.6	14.7 - 29.7	14.9 - 30.1	14.7 – 30.4
Salinity (‰)	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0
Ammonia (mg/L)	0.17 \pm 0.01	0.17 \pm 0.01	0.17 \pm 0.01	0.17 \pm 0.01
Nitrite (mg/L)	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0	0.01 \pm 0.0
Alkalinity (mg/L)	49.1 \pm 0.60 ^c	46.6 \pm 0.35 ^{ab}	45.4 \pm 0.53 ^a	48.0 \pm 0.72 ^b
Phosphorus (mg/L)	0.19 \pm 0.0 ^b	0.18 \pm 0.0 ^a	0.18 \pm 0.0 ^a	0.19 \pm 0.0 ^b
Calcium (mg/L)	7.90 \pm 0.22 ^c	7.23 \pm 0.22 ^b	6.34 \pm 0.20 ^a	7.59 \pm 0.23 ^b
Secchi disk (cm)	30.6 \pm 0.9	31.6 \pm 0.9	31.7 \pm 1.0	32.5 \pm 1.0

ANOVA, N=1008, pH: $F_{3, 1004}=2.923$, $P=0.033$; Conductivity: $F_{3, 1004}=0.258$, $P=0.856$; Oxygen-mean : $F_{3, 1004}=1.425$, $P=0.234$; Temp-mean: $F_{3, 1004}=0.817$, $P=0.485$; Salinity: $F_{3, 1004}=0.199$, $P=0.897$; N=144, Ammonia: $F_{3, 140}=0.243$, $P=0.867$; Nitrite: $F_{3, 140}=1.113$, $P=0.346$; Alkalinity: $F_{3, 140}=8.249$, $P<0.001$; Phosphorus: $F_{3, 140}=5.533$, $P=0.001$; Calcium: $F_{3, 140}=9.693$, $P<0.001$; Secchi disk: $F_{3, 248}=0.666$, $P=0.574$.

Note: Values with different superscripts in a row are significantly different (ANOVA, $P<0.05$).

3.4 Discussion

Well fertilized ponds normally have levels of high protein natural food (Shroeder, 1983; Hepher, 1988) and often a mixture of organisms containing 50-60% protein on a dry weight basis (De Silva, 1993). It is, therefore, important to complement the natural high protein diet with locally available cheap energy feed (Edwards *et al.*, 1988). The supplementation in this experiment had a significant influence on the growth of fish in fertilized ponds. Fish fed soybean meal grew faster than those fed maize bran, rice bran supplements and chicken manure pond fertilization. They had significantly higher final mean weights compared to maize bran, rice bran and chicken manure treatments.

Except for work in Asia and the United States, little work has been done with soybean use as a single supplement in ponds under semi-intensive systems (Tacon, 1988; De Silva, 1993, Robinson and Li, 1994; Cremer *et al.*, 2000) and information is not readily available for other tropical locations. Chikafumbwa (1996a) reported supplementation of napier grass with maize bran in ponds stocked with *T. rendalli* and *O. shiranus* and the mean final weights were 37.6 g and 35.5 g from initial weight of 9.2 g and 9.3 g, respectively in 181 days. This represented a percent increase of 309% for *T. rendalli* and 283% for *O. shiranus*. Those results were lower than 319% increase in the chicken manure only treatment in this experiment. Chikafumbwa (1996a) also reported lower specific growth rates than found in the present study. However, when napier grass was processed by chopping and combined with maize bran, specific growth rates improved to 1.46 %/day. This was within the range of the present results with maize bran supplementation but lower than growth rates realised with soybean fed fish (2.17 %/day).

Liti *et al.* (2002) reported higher weight gains (0.90 g/day) in fertilized ponds stocked with *T. niloticus* and *Clarias gariepinus* fed rice bran than those (0.22 g/day) in the present experiment. This could be due to different species of fish as *T. niloticus* tend to grow faster (Yi and Kwei Lin, 1996; Beniga and Circa, 1997) than *T. rendalli* and the rice bran used in their experiment had higher lipid than in that used in this experiment among others.

Maize bran was also found to produce better growth rates than rice bran when fed to *O. shiranus* in monoculture. However, when both *O. shiranus* and *T. rendalli* were given rice bran in monoculture, *T. rendalli* showed better growth rates and feed conversion ratios than *O. shiranus* in fertilized tanks (Ohashi *et al.*, 1999). There were differences among the species of fish in acceptability and utilization of feed. The differences could be due to form of the feed, as most people use powder form (De Silva, 1993). There are also variations in the quality of these feeds in terms of nutritive value (Bardack and Santerre, 1979; NRC, 1993), which is different from one ingredient supplement to the other. Maize bran produced better growth rates than rice bran in the present experiment probably because of the low crude fibre in maize bran compared to rice bran (Shiau and Chang, 1995), since the protein levels were comparable.

Fish cultured in ponds supplied with soybean and maize bran had lower feed conversion ratios than did those in ponds supplied with rice bran. The lower conversion ratios meant better feed utilization and were also reflected in feed conversion efficiencies which were higher in soybean and maize bran than with rice bran. Overall, conversion ratios in the present experiment were comparable to those reported in similar studies

(Diana *et al.*, 1996; Fitzsimmons *et al.*, 1999; Yi *et al.*, 2002). However, high feed conversion ratios (8.0-10.5) were reported in *O. niloticus* and *C. gariepinus* polyculture when fed rice bran in fertilized ponds (Veverica *et al.* 2000) and 5.26-9.55 in a cyprinid, Mahseer, *Tor putitora* (Hamilton) raised in ponds fed formulated supplementary diets (Islam, 2002). Ohashi *et al.* (1999) also reported slightly higher conversion ratios (2.15-5.49) than in the present experiment in *O. shiranus* and *T. rendalli* in monoculture and polyculture systems fed maize bran and rice bran in fertilized tanks. Similar conversion ratios were reported earlier by Tacon (1988) with soybean (3-5), corn (4-6), cereals (4-6) and groundnut cake (2-4). The differences could be attributed to the species of fish used, processing and presentation of feed (Hasan and Amin, 1997; Edwards *et al.*, 2000). Significant waste of feed is experienced where feeding is done in powder form (De Silva, 1993; Miaje *et al.*, 1999) and can result in higher conversion ratios. In the present experiment, the feeds were in pellet form though the pellets from rice bran were unstable compared to soybean and maize bran due to rice bran's nature of poor pelletability. This may have contributed to significantly higher feed conversion ratios in rice bran treatment than those from soybean and maize bran treatments in this experiment. Feeding frequencies, which vary with species, could also have influenced the conversion ratios (Hasan, 2001) in these studies.

The extrapolated annual yields (kg/ha/yr) were higher in soybean meal fed fish than what was reported by other researchers in similar experiments (Noble and Chimatiro, 1991; Chikafumbwa, 1996b; Brummett, 2000; Veverica *et al.*, 2000; Chaula *et al.*, 2002). Their results were within the range of yields obtained in the maize bran, rice

bran supplementation and chicken manure in the present experiment. Yakupitiyage *et al.* (1991) reported high yields with Nile tilapia, common carp and silver barb fed rice bran at different rates in integrated system with ducks. Intensification level in the integrated system can influence yield as there is evidence that even minor modifications of inputs into semi-intensive systems can bring about changes in terms of growth, reproductive performance and overall productivity of the system (De Silva and Davy, 1993; Vareena *et al.*, 1993; Berkan, 1996, Tacon and De Silva, 1997; Miaje *et al.*, 1999; Keshavanath *et al.*, 2002).

The survival of the fish in this experiment was higher than in several similar studies (Chikafumbwa, 1996b; Brooks and Maluwa, 1997; Veverica *et al.*, 2000; Brown *et al.* 2002; Islam, 2002) but comparable to those reported by Liti *et al.* (2002) and Chaula *et al.* (2002). This could be attributed to low predation, reduced handling stress and suitable water requirements experienced during the experiment. Temperature and dissolved oxygen together with other parameters were within range for the growth of fish, and for various species of phytoplankton and zooplankton to propagate in ponds, to act as food for fish (Delbare and Dert, 1996).

The type of supplementary feed used in this experiment also had significant effects on the body composition of *T. rendalli*. There was an increase in fat and gross energy content, with a decrease in ash and protein content. These trends are common in fed fish as they store more fat and gross energy with high-energy diets containing such ingredients (Maraise and Kissil, 1979; Hanley, 1991; Chimoka, 1998). Moisture decreased with soybean but increased significantly with maize bran, rice bran and

chicken manure. Researchers working with fish in ponds seldom report the biochemical composition of their fish. Yi *et al.* (2002) reported higher moisture and ash content in fish fed different species of phytoplankton than in the present experiment; however, protein and fat content were within the same range. The similarities and differences encountered could be attributed to the fact that the fish in their studies were cultured in a saline environment where energy demands are different from freshwater, which might have affected the utilization of the feeds supplied, resulting in different biochemical body composition.

Natural biological productivity in terms of plankton abundance was affected by supplementation. Soybean fed ponds had significantly higher numbers of copepods, especially *Cyclops*. Cladocerans of the *Moina* genus and rotifers of the *Lecane* genus were also higher in the soybean treated ponds. Zooplankton numbers were higher than those reported in other organic fertilized pond experiments in similar systems (Geiger, 1983; Brummett, 2000a; Dhawan and Kaur, 2002) but consistent with those reported by Kamanga and Kaunda (1998). The amount of chlorophyll *a* was higher in the soybean meal treated ponds than in maize bran, rice bran and chicken manure treatments. The increase in chlorophyll *a* could be due to the high fertilization effect of uneaten proteineous soybean, which is high in nitrogen (Tacon, 1990; NRC, 1993; El-Sayed, 1999; Alceste, 2000) that enhanced the fertility and growth of phytoplankton (algae) in ponds (Swift, 1993; Tacon and De Silva, 1997). Algae have high protein, fat, and ash content (De Silva, 1993; Siwick, 2001), which could have influenced the growth and composition of both the fish and the zooplankton.

The biochemical composition of zooplankton as food for fish varied among treatments in terms of moisture, ash, fat, protein and gross energy content. Zooplankton in soybean fed ponds had significantly lower moisture and gross energy content but was higher in ash, fat and protein content, and these were consistent with the composition reported for rotifers, cladocerans and copepods on a dry weight basis (Watanabe, 1988; De Silva, 1993; Delbare *et al.*, 1996). The variation in their biochemical composition could be attributed to the amount of phytoplankton available in the ponds, as the same treatment ponds had high amount of chlorophyll *a*. The availability of phytoplankton as food for zooplankton, must have influenced the quality of their body composition thus influencing the composition of the fish as discussed above.

The stomach contents of the fish reflected the type and amount of food that was available in the culture ponds and included detritus, higher plants, zooplankton, insects and feed. The feed was the least amount of the content in the stomachs and this could be due to fast clearance rate in the gut as they were fed twice a day and digestion could be faster than the natural food, which was always present in large amounts in the pond. Fish cultured with rice bran supplement and chicken manure fertilization consumed significantly more detritus but higher plants were greater in fish in soybean and maize bran treatments. There were variations in the consumption of phytoplankton and zooplankton among treatments but there was clear indication that *T. rendalli* consumed a substantial amount of phytoplankton and zooplankton as opposed to what was reported by Brummett (2000a), where stomach contents were almost free from plankton in fish between 36-40 g. His results were, however, consistent with the consumption of detritus

and higher plants in fish of similar weight as in the present experiment. There is evidence that many tilapias feed on plant matter and plankton (Lowe, 1958; Moriarty, 1973; Bowen, 1982) and their feeding habits may change at different life stages and environments in which the fish are cultured (Jauncey, 1998).

There were no significant differences in pH, conductance, mean dissolved oxygen, and mean temperatures, salinity, ammonia, nitrite and secchi disk visibilities of the water among the treatments. The levels were within the ranges for general tilapia requirements (Philippart and Ruwet, 1982; Buttner *et al.*, 1993; Boyd and Turker, 1998) and were consistent with other reported research work in similar systems (Chikafumbwa, 1996b; Brummett, 2000a; Veverica *et al.*, 2001; Chaula *et al.*, 2002). The ponds however, experienced lower dissolved oxygen in the morning but improved during the day. The low oxygen levels in the morning are common in fertilized ponds as oxygen is depleted at night (Knud-Hansen, 1998).

There were significant differences among the water minerals and alkalinity levels but they were within range for pond culture of similar systems (Brooks and Maluwa, 1997). Alkalinity was above 20 mg/l, which is conducive for the growth of phytoplankton and other organisms (Boyd, 1982; Boyd 1990; Boyd and Tucker, 1998). The loading of organic matter was also consistent with those reported by Brummett (2000a) and Boyd (1990) in similar systems. Organic matter and detritus facilitate growth of microorganisms (Geiger, 1983; Moriarty, 1997) and release nutrients for phytoplankton growth that is consumed by fish and zooplankton in food webs present in many pond

ecosystems. Fish can also consume the manure directly with microorganisms, which is high in nutritive value.

3.5 Conclusion

Fish grew better in ponds supplied with soybean meal than those cultured with maize and rice bran supplements and chicken manure alone. In many aspects rice bran and chicken manure produced significantly lower results compared to the rest of the treatments. The growth rates, yields, natural production (zooplankton and phytoplankton) in ponds treated with soybean performed best. The performance of fish fed maize bran and rice bran differed significantly but was well above only chicken manure fertilization.

There was a demonstration of increase in yields of fish with supplementation. Yield increased due to supplementation by 5% with rice bran, 27.8% with maize bran and 76.8% with soybean meal. It appears advantageous to supplement fertilized ponds to increase yields. The choice of supplementary feed would depend on availability and cost, as protein rich feeds are more expensive than those rich in carbohydrates. High quality supplements like soybeans would be more expensive than maize bran and rice bran. Choices have to be made basing on the current situation. Farmers may be advised to take advantage of obtaining soybean just after harvesting, as the cost is cheaper during this period because the supply is high. Dilution of protein rich feeds with carbohydrates to make simple mixtures of feed could be an option to improve the nutrition of the feeds and reduce overall feed cost (Hepher, 1990; Sumagaysay *et al.*, 1990).

Chapter 4

EFFECT OF TEMPERATURE ON GROWTH, FEED UTILIZATION AND SURVIVAL OF *TILAPIA RENDALLI* IN AQUARIA.

4.1 Introduction

Tilapia are thermophilic fishes and their geographic distribution is closely determined by temperature, particularly low temperatures (Philippart and Ruwet, 1982; Pauly, 1998). In general, tilapia growth and reproduction cease at temperatures below 20° C (Philippart and Ruwet, 1982; Huet, 1994). However, their accretive thermal range varies from 30 to 36° C (Caulton, 1982). Food intake is related to both body mass and environmental temperature, and assimilation efficiency increases with increase in temperature, including metabolism (Caulton, 1982). Temperature has been considered the major environmental factor influencing the physiological functions of many fish species (Marcicondi-Massari *et al.*, 1998; Ridha *et al.*, 1998; Costa *et al.*, 2000; Mallekh and Lagardere, 2002; Atwood *et al.*, 2003).

Among the tilapia species grown in Malawi, *Tilapia rendalli* is one of the most important. However, there is lack of understanding of the basic factors that affect their growth. As a result many farmers who raise these fish experience low production. This fish is favoured by many farmers throughout the country and has the potential of expressing good growth characteristics if their culture conditions are improved. Malawi is classified as falling within the sub humid, semi-arid zone of southern Africa characterised by three seasons: a hot and wet season (November-March), a cool and dry season (April-August) and a hot dry season (September-November) (Dickson and Brooks, 1997). The temperatures experienced in Malawi vary with its agro-ecological areas. In summer, the south is characterised by high temperatures (>30° C) (Internet Africa, 1997) while the centre experiences moderate temperatures (25-30° C) and low temperatures (20-25° C)

are experienced in the northern part of the country. These temperature variations affect water temperature and that in turn affects the growth and reproductive performance of both tilapia and catfish (Brooks and Maluwa, 1997). This information suggests that temperatures would have an effect on the growth of *T. rendalli* in their natural habitats, where this would also affect the utilization of feed under such conditions (Caulton, 1982). There is little or no information on *T. rendalli* fed formulated all-plant diets raised under different water temperatures. The concentration has been on coldwater fish species (Martinez-Palacios *et al.*, 2002) where temperature variations that affect the growth performance of fish may not be as great as experienced in the tropics. This experiment was, therefore, set up to assess the effect of different water temperatures on the growth, feed utilization and survival of *T. rendalli* under laboratory conditions.

4.2 Material and methods

4.2.1 Experimental facilities and experimental set-up

The experiments were conducted in the laboratory facility at the Aquaculture experimental farm of the Department of Aquaculture and Fisheries Science at Bunda College of Agriculture. Twelve 50 L rectangular glass tanks were set up in the wet laboratory (Fig. 4.1). A heater (200 W) and a thermometer were placed in each tank and experimental temperatures were reached by increasing the temperature by 2° C every day from 20° C. A bio-filter (Matsumoto) was put in the tank to help clean the water. Four temperature treatments (24, 28, 32° C and ambient temperature) were assigned to the

tanks in a completely randomised design (CRD), having three replicates in each treatment. The experiment was carried out under ambient photoperiod.

4.2.2 Experimental animals and stocking

T. rendalli juveniles of mixed sex were collected from the breeding ponds of the experimental farm by netting. They were then held in a bigger tank in the hatchery building for acclimation for one week. During this period, the fish were given maize bran for their maintenance prior to the commencement of the laboratory experiment. They were transferred and placed in the 50 L experimental tanks filled with well water and stocked at the rate of 15 fish per tank. Aeration was provided throughout the experimental period. There was a one-week acclimation prior to the start of the experiment and during this period any dead fish were replaced with fish of similar sizes. After acclimation, the initial weight and total length of fish were taken and they averaged 6.64 ± 1.03 g and 7.06 ± 0.44 cm, respectively. Water was changed every morning at the rate of 40 - 50% with water at similar temperatures and tanks were also cleaned at this time. During sampling, all the fish in each tank were weighed and measured as described in the previous Chapters.



a)



b)

Fig. 4.1. The set up of all tanks (a) and individual tank (b) in the wet laboratory where *Tilapia rendalli* were cultured at different temperatures.

4.2.3 Feeds and feeding

4.2.3.1 Acquisition of feed ingredients used in the experiment

In fish nutrition, ingredients used in practical fish feeds can be classified as protein (amino acid) sources, energy sources, essential lipid sources, vitamin supplements, mineral supplements and special ingredients to enhance growth, pigmentation, sexual development in fish, palatability of diet or preservation of the feeds. Ingredients used included maize bran and soybeans, cotton seed cake, rice, vitamin and mineral supplements, and wheat flour. Maize bran and soybeans were obtained from villages around Bunda College, cotton seed cake from Mbado manufacturing Industries in Blantyre City and rice bran from the Govala market in Zomba. Cottonseed oil (Kukoma) and wheat flour (a binder) were obtained from shops around Lilongwe city. Vitamin and mineral supplements were obtained from commercial laboratories within Malawi.

4.2.3.2 Proximate analysis of feed ingredients

The ingredients were ground and passed through a 0.2 mm sieve before they were assayed for proximate analysis using standard methods (AOAC, 1990) for dry matter, crude protein, crude fat, crude fibre, ash. Other characteristics of the ingredients such as nitrogen free extract, organic matter, available lysine, trypsin inhibitor, gross energy and minerals were also analysed or calculated as outlined in section 3.2.2 (Chapter 3) (Table 4.1).

Table 4. 1 Proximate analysis of the ingredients used for the formulation of experimental diet fed to *Tilapia rendalli* under different temperature conditions (dry weight)(Mean \pm SD).

Component analysed (%)	Soybean	Cotton seed cake	Maize bran	Rice bran
Dry Matter (DM) ¹	93.3 \pm 1.9	92.7 \pm 0.8	91.8 \pm 0.6	91.5 \pm 0.5
Crude Protein (CP)	42.1 \pm 1.3	42.0 \pm 1.4	13.4 \pm 0.6	12.8 \pm 0.2
Crude Fibre (CF)	6.13 \pm 0.41	11.5 \pm 0.35	3.7 \pm 0.10	11.3 \pm 0.04
Crude Fat	20.9 \pm 0.7	2.2 \pm 0.2	10.1 \pm 0.5	7.1 \pm 0.4
Ash	4.5 \pm 0.2	6.6 \pm 0.2	3.3 \pm 0.3	10.7 \pm 0.6
Phosphorus	0.57 \pm 0.03	1.14 \pm 0.05	0.51 \pm 0.02	0.21 \pm 0.02
Calcium	0.10 \pm 0.04	0.17 \pm 0.02	0.03 \pm 0.01	0.02 \pm 0.01
Potassium	0.47 \pm 0.05	1.34 \pm 0.06	0.37 \pm 0.04	0.12 \pm 0.01
NFE	26.5 \pm 1.5	37.7 \pm 1.1	69.5 \pm 0.5	58.2 \pm 0.2
Organic matter	88.8 \pm 0.7	86.1 \pm 0.6	88.5 \pm 0.2	80.9 \pm 0.1
Available lysine (%)	4.62 \pm 1.12	4.77 \pm 0.08	-	-
Trypsin Inhibition (%)	44.1 \pm 2.6	-	-	-
Gross Energy (kJ g ⁻¹)	20.9 \pm 0.1	19.5 \pm 0.1	18.5 \pm 0.4	16.3 \pm 0.1

- Not analysed; ¹ % wet weight; NFE-Nitrogen free extract.

4.2.3.3 Diet formulation

The diet used in the experiment was formulated using a computer program, linear programming (MIXIT.WIN), provided by Agricultural Software Consultants, Inc. (1997). The diet was an all-plant diet formulated to contain 30% crude protein. Studies have shown that smaller tilapia could grow well with 25% or less crude protein. Smaller

channel catfish grow well with a 27% protein diet (Lovell, 1989). The 30% crude protein level in the present study was well above the requirement for the culture of *T. rendalli* (Table 4.2).

4.2.3.4 Diet preparation

Soybeans were roasted until the point of browning and cracking. Soybeans, maize bran, rice bran and cottonseed cake were milled to pass through a 0.2 mm sieve. Other ingredients, like bread flower, vitamin and mineral supplement were already in powder form. The ingredients were mixed by hand to have the desirable mixture before pelleting. Water (about 500 ml) was added to each kg of mixture to make a dough for pelleting. A hand mincer was used as a pelleter to make 2 mm pellets. The pellets were then airdried to moisture of less than 10% and frozen until used. The feed preparation procedure was similar to that outlined by Royes and Chapman (2003).

4.2.3.5 Diet analysis

The diets were assayed for proximate analysis of dry matter, crude protein, crude fat, ash and gross energy using standard methods (AOAC, 1990) as outlined in section 3.2.2 (Chapter 3). Other characteristics of the diets, such as organic matter and nitrogen free extract, were calculated by difference (El-Sayed, 2003) (Table 4.3).

Table 4.2 Composition of experimental diet fed to *Tilapia rendalli* cultured at different temperature conditions for 70 days.

Ingredient	% dry weight
Maize Bran (MB)	22.00
Rice Bran (RB)	13.45
Cotton Seed Cake (CSC)	24.55
Soy Bean Meal (SBM)	35.00
¹ Vitamin Premix	1.00
² Mineral Premix	1.00
Oil (Cotton Seed)	2.00
Wheat flower (Binding material)	1.00
Total	100.00

¹**Vitamin Premix (g/100 g of premix) contained :** retinol (vit A), 2,000,000 iu; d pantothenic acid (vit B6), 100 mg; riboflavin (vitB12), 500 mg; thiamine (vit B1), 300 mg; vit E. 1,00 iu; vit K, 500 mg; folic acid 50 mg; niacin 2500 mg; ascorbic acid (vit C), 500 mg; vit D3, 245,000 iu; pyrodoxine,100mg.

²**Mineral Premix (g/100g of premix) contained:** magnesium sulphate, 50.00; sodium sulphate, 30.00; potassium chloride, 5.00; calcium hydrogen orthophosphae, 10.29; zinc sulphate, 2.7; cobalt sulphate 0.24; copper sulphate, 0.39; chromic chloride; 0.064; manganese sulphate, 1.27. Sodium Chloride,30.00.

Table 4.3 Proximate composition of the experimental diet fed to *Tilapia rendalli* cultured at different temperature conditions for 70 days (dry weight) (Mean \pm SD).

Proximate Composition	%
Dry matter ¹	89.08 \pm 1.09
Crude protein	30.69 \pm 0.97
Crude fat	11.09 \pm 0.32
Ash	8.91 \pm 0.92
Crude fibre	6.13 \pm 0.42
NFE	43.18 \pm 1.64
Organic matter	80.17 \pm 0.67
Gross energy (kJ g ⁻¹)	19.54 \pm 1.35

¹ Wet weight

4.2.3.6 Fish feeding

The diets were prepared before the commencement of the experiment and were frozen. Feeding was done twice a day at a ration of 3% body weight. This meant that 1.5% was applied at 08:00 hr and the other 1.5% at 14: 00 hr. Hand feeding was done to spread the pellets (Fig. 4.2) evenly on top of the water in the tank. Left-over feed was siphoned every morning, dried to constant weight in an oven at 105° C and weighed. The daily feed intake was determined (Bhikajee and Gobin, 1997). Feces that accumulated at

the bottom of the tanks were siphoned out every morning during the cleaning process, before feeding commenced.

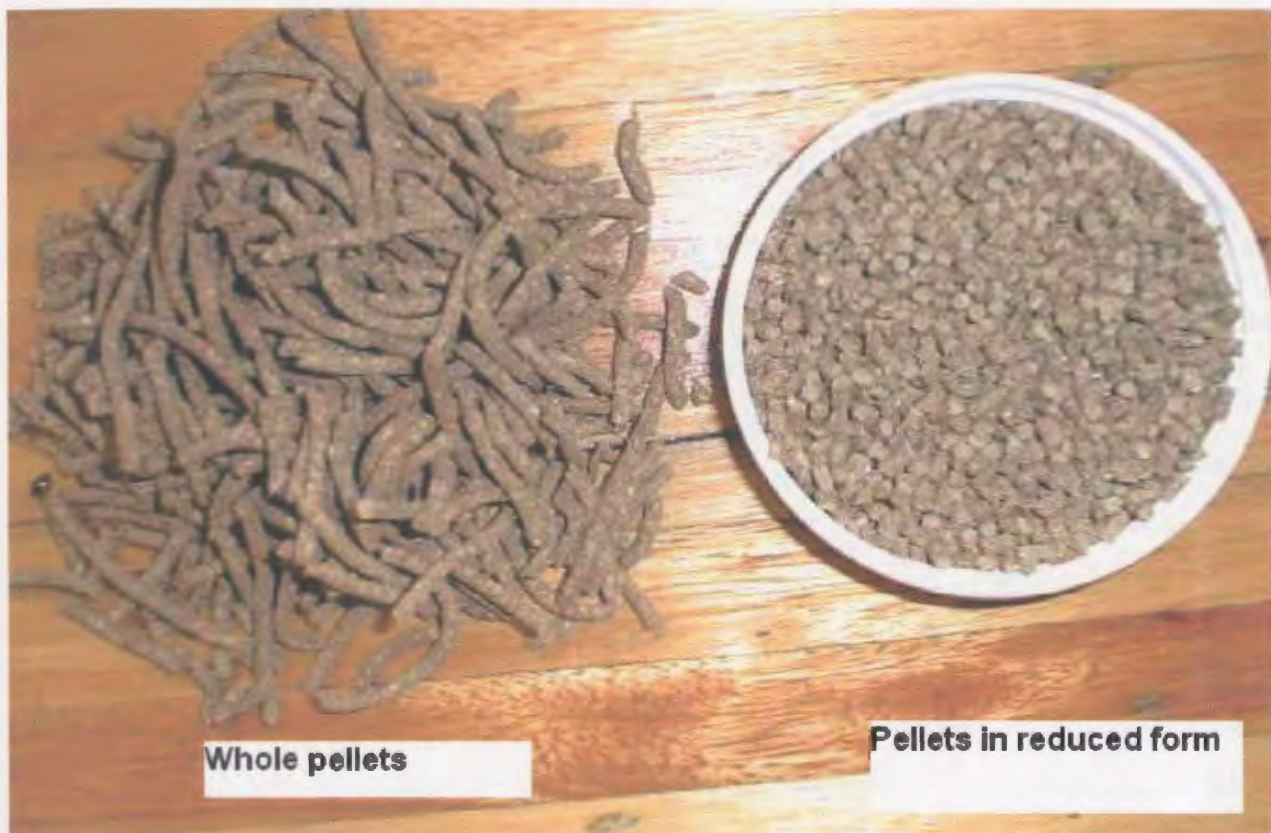


Fig. 4.2. Pellets (whole and reduced form) fed to *Tilapia rendalli* under different temperature conditions.

4.2.4 Fish weighing and data collection

4.2.4.1 Fish weighing

Weighing of the fish was done every two weeks as in the previous Chapters. The feed given was adjusted after weighing the fish during each sampling and the tanks were thoroughly cleaned.

4.2.4.2 Fish growth performance

Data collected include total body weight (g) and total lengths (cm). Fish were counted daily to monitor survival. Parameters of fish growth performance included weight gain, percentage (%) increase in average weight, specific growth rate (%/day) and were calculated using the formulae outlined section 2.2.5 (Chapter 2). The daily feed consumption was calculated as follows (Watanabe, 1988):

Daily feed consumption (%) = feed intake (g) / (initial body wt + final body wt) / (2 x period (days)) x 100

4.2.4.3 Growth performance index

Indices for performance of the fish were calculated using the following formulae:

- a) Gonado-somatic index (GSI) (%) = weight of gonads (g) / weight of fish (g) x 100.
- b) Gastro-somatic index (GI) (%) = weight of gastrointestinal tract (g) / weight of fish (g) x 100.
- c) Thermal unit growth coefficient = {(final body wt)^{1/3} - (initial body wt)^{1/3}} / degree days (° C)x 100

- d) Fulton's condition factor was calculated using the formula as outlined in section 2.2.5 (Chapter 2).

4.2.4.4 Feed and protein utilization

Feed utilization was calculated using the relationship between the total amount of food provided and the body mass attained as feed conversion ratio (FCR) and feed conversion efficiency (FCE). The formulae used were as outlined in section 3.2.8 (Chapter 3). Protein utilization (PER) was calculated as the ratio between weight gain (g) and protein intake (g).

4.2.4.5 Analysis of fish body composition

The analysis of fish samples was done prior to the commencement and at the end of the experiment for moisture, crude protein, crude fat, ash and gross energy. The procedures followed were as described in section 2.2.7 (Chapter 2).

4.2.5 Digestibility determination

Digestibility describes the fraction of the nutrient or energy in the feedstuff that is not excreted in the feces. The indirect method was used to determine the digestibility of the diet in fish in this experiment. This involved incorporation of an inert marker, chromic oxide (Cr_2O_3) into the diet at 1%. The trial was conducted for 14 days after the 70-day growing period. Eight fish from the experiment were used in each tank to monitor digestibility. The fish were fed the diet containing chromic oxide for 5 to 7 days before

feces collection began. Feces were collected by siphoning for one week, every four hours after feeding. The feces collected were pooled from each treatment and stored in a refrigerator. They were dried at 105° C and analysed for crude protein, ash, fat and gross energy using standard methods (AOAC, 1990) and chromic oxide was analysed using the procedure by Furukawa and Tsukahara (1966). The digestibility coefficients for nutrients and energy were calculated using the following formula (NRC, 1993):

$$\text{Digestion coefficient (\%)} = 100 - 100 \frac{(\% \text{ marker in feed})(\% \text{ nutrient in feces})}{(\% \text{ marker in feces})(\% \text{ nutrient in feed})}$$

4.2.6 Water quality monitoring

Temperature (° C), dissolved oxygen (mg/L), pH, and electrical conductivity (µS cm⁻¹) were measured every day at 08:00 hr and 14:00 hr throughout the culture period. Ammonia (mg/L) and nitrite (mg/L) were measured once a week using the titration method as described in section 2.2.10 (Chapter 2).

4.2.7 Data collection and statistical analysis

The data collection was as in Chapter 2. The analysis was performed to test the effect of different temperature levels on the performance of fish. Regression analysis was performed on the final weight and temperature to assess their relationship. All statistical analyses were carried out using SPSS 10.0 (SPSS Inc., 1999) and presentation of the data was as described in section 2.4 (Chapter 2).

4.3 Results

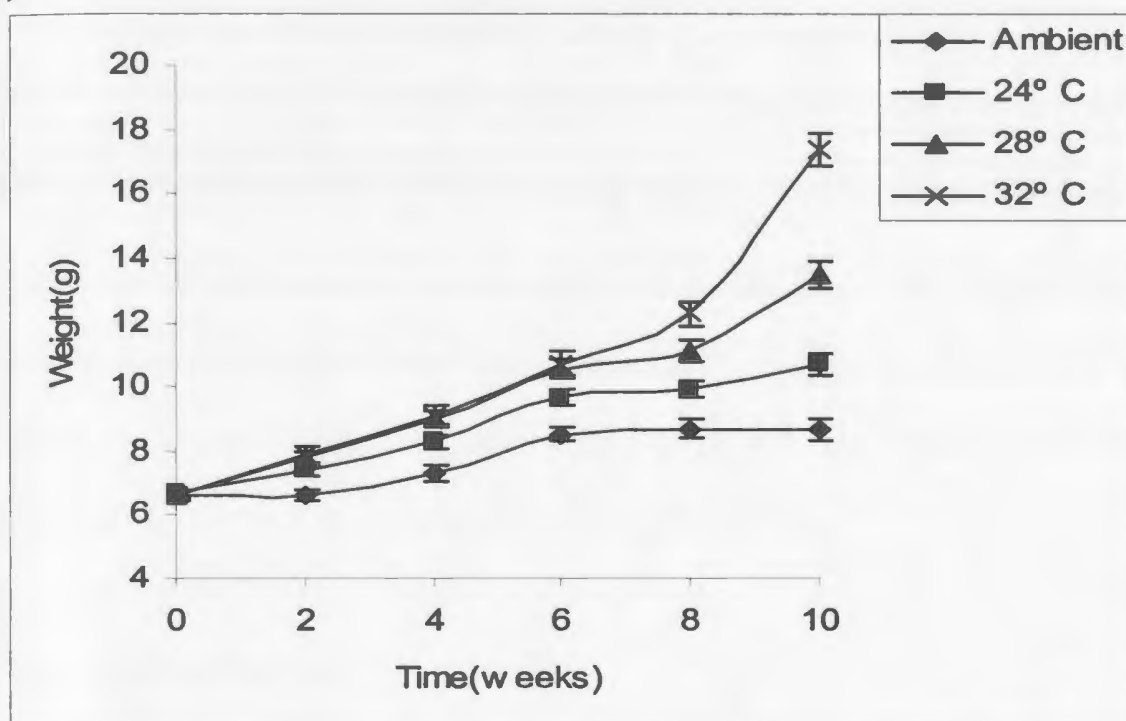
4.3.1 Body weight

Data met the assumptions of ANOVA. Initial weights were not significantly ($P>0.05$) different. Individual body weights started to show significant ($P< 0.05$) differences two weeks after stocking (Fig 4.3). Fish in the ambient temperature treatment had significantly lower average weights in the first sampling than the rest of the treatments, which did not differ significantly during this time. Fish cultured at 32° C performed better than the rest of the treatments and attained final average weights of 17.37 g, compared to 10.7 g and 13.49 g for 24° C and 28° C, respectively. Fish in the ambient temperature treatment had significantly lower average weights throughout the experiment (Fig. 4.3). Growth of fish generally increased with temperature. Linear regression analysis indicated a significant ($F_{1,178} = 227.03$, $P< 0.001$) and strong ($R^2 = 0.6088$) relationship between the final weight and temperature with the following equation: $\text{weight} = 0.761 \text{ temperature} - 7.3482$ (Fig 4.4; Appendix 4.1).

4.3.2 Growth characteristics, condition and survival of fish

Total weight gains (initial weight - final weight) were significantly ($P< 0.05$) different among treatments. Higher total weight gains were found in fish cultured in the 32° C treatment (10.75 g) compared to those in ambient (2.00g), 24° C (4.01 g) and 28° C (6.89 g). The weight gain per day and percent increase in weight over the experimental period showed the same trend of total weight gain. Significantly higher weight gain per day and the percent increase was observed in fish cultured at 32° C treatment (Table 4.4).

a)



b)

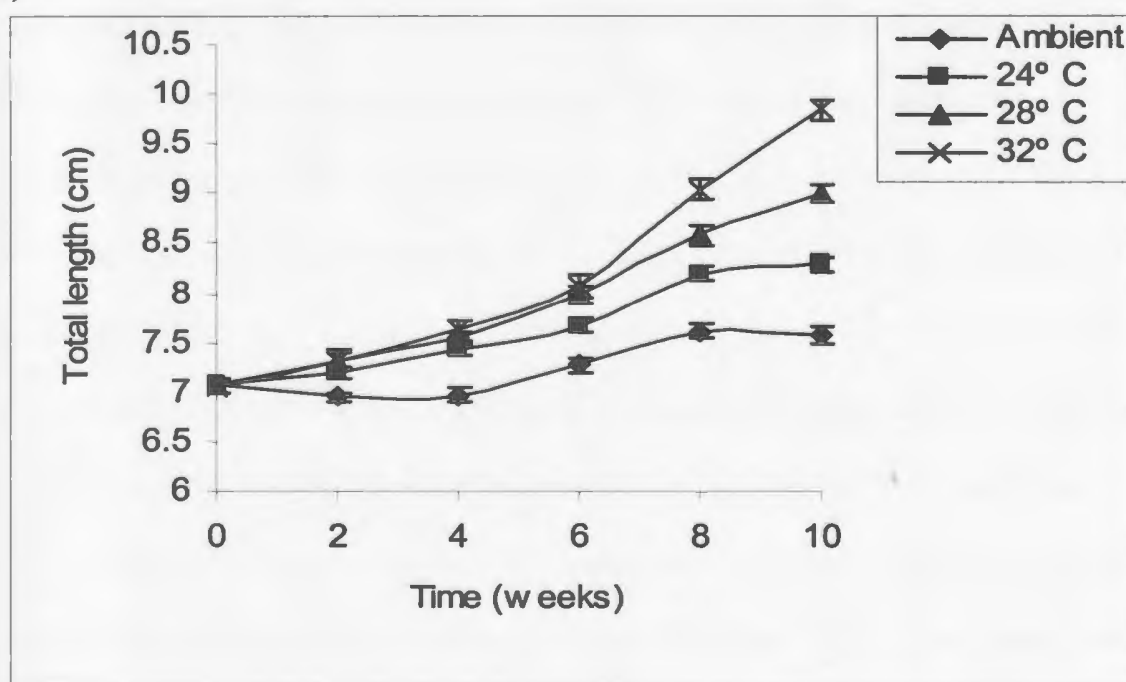


Fig. 4.3. Mean (\pm SE) of weight (a) and total length (b) of *Tilapia rendalli* cultured at different temperatures for 70 days (N=45).

Specific growth rates (SGR) were significantly ($P < 0.05$) different among treatments. The growth rates were temperature dependent and the lowest rates were in the ambient temperature (0.35 %/day) and the highest at 32° C (1.37 %/day). The initial condition factor of the fish did not differ significantly among treatments. However, final condition factors differed significantly with temperature. Fish cultured at ambient temperature had a significantly better condition factor than the fish in the rest of the treatments, which did not differ significantly (Table 4.4). There was a 100% survival rate throughout the experimental period across all treatments.

4.3.3 Feed utilization

The highest total amount of feed was consumed by the fish grown at 32° C treatment (815 g) and the lowest in fish cultured at ambient temperature (534 g). The amount of food consumption was temperature dependent, increasing with increasing temperature. There were significant ($P < 0.05$) differences among treatments in the amount of feed intake in relation to body size and time of culture (%/day) (Table 4.5). Fish cultured at 32° C and those from ambient temperature treatments did not differ significantly in their percent feed intake per day, but their feed intake was lower than fish cultured at 24° C and 28° C, which did not differ significantly from each other.

Feed conversion ratios (FCR) were also temperature dependent and differed significantly among treatments. Feed conversion ratios were significantly lower in fish cultured at 32° C (1.78) followed by fish at 28° C (2.8) and at 24° C (4.71) (Fig. 4.5).

Table 4.4 Initial weight, final weight, weight gain, weight gain per day, weight increase, specific growth rate (SGR), initial condition, final condition and survival of *Tilapia rendalli* cultured at different temperatures (Mean \pm SE).

Parameter	Treatment			
	Ambient	24° C	28° C	32° C
Initial wt (g)	6.66 \pm 0.27	6.69 \pm 0.14	6.60 \pm 0.16	6.62 \pm 0.16
Final weight (g)	8.65 \pm 0.32 ^a	10.70 \pm 0.32 ^b	13.49 \pm 0.42 ^c	17.37 \pm 0.47 ^d
Weight gain (g)	2.00 \pm 0.37 ^a	4.01 \pm 0.33 ^b	6.89 \pm 0.45 ^c	10.75 \pm 0.52 ^d
Weight gain/day	0.03 \pm 0.01 ^a	0.06 \pm 0.01 ^b	0.09 \pm 0.01 ^c	0.15 \pm 0.01 ^d
Weight increase (%)	33.9 \pm 6.33 ^a	62.5 \pm 5.8 ^b	109.6 \pm 8.5 ^c	170.2 \pm 10.7 ^d
SGR (%/day)	0.35 \pm 0.06 ^a	0.66 \pm 0.05 ^b	1.01 \pm 0.56 ^c	1.37 \pm 0.06 ^d
Initial condition	1.88 \pm 0.03	1.90 \pm 0.03	1.87 \pm 0.03	1.88 \pm 0.02
Final condition	1.96 \pm 0.03 ^b	1.85 \pm 0.02 ^a	1.82 \pm 0.02 ^a	1.81 \pm 0.02 ^a
Survival (%)	100	100	100	100

ANOVA, N=180, Initial weight: $F_{3, 176} = 0.069$, $P = 0.976$; Final weight: $F_{3, 176} = 92.917$, $P < 0.001$; Weight gain: $F_{3, 176} = 79.557$, $P < 0.001$; Weight gain per day: $F_{3, 176} = 79.557$, $P < 0.001$; Weight incr: $F_{3, 176} = 54.389$, $P < 0.001$; SGR: $F_{3, 176} = 61.523$, $P < 0.001$; Initial condition: $F_{3, 176} = 0.233$, $P = 0.873$; Final condition: $F_{3, 176} = 8.166$, $P < 0.001$;

Note: Values with different superscripts in a row are significantly different (ANOVA, $P < 0.05$)

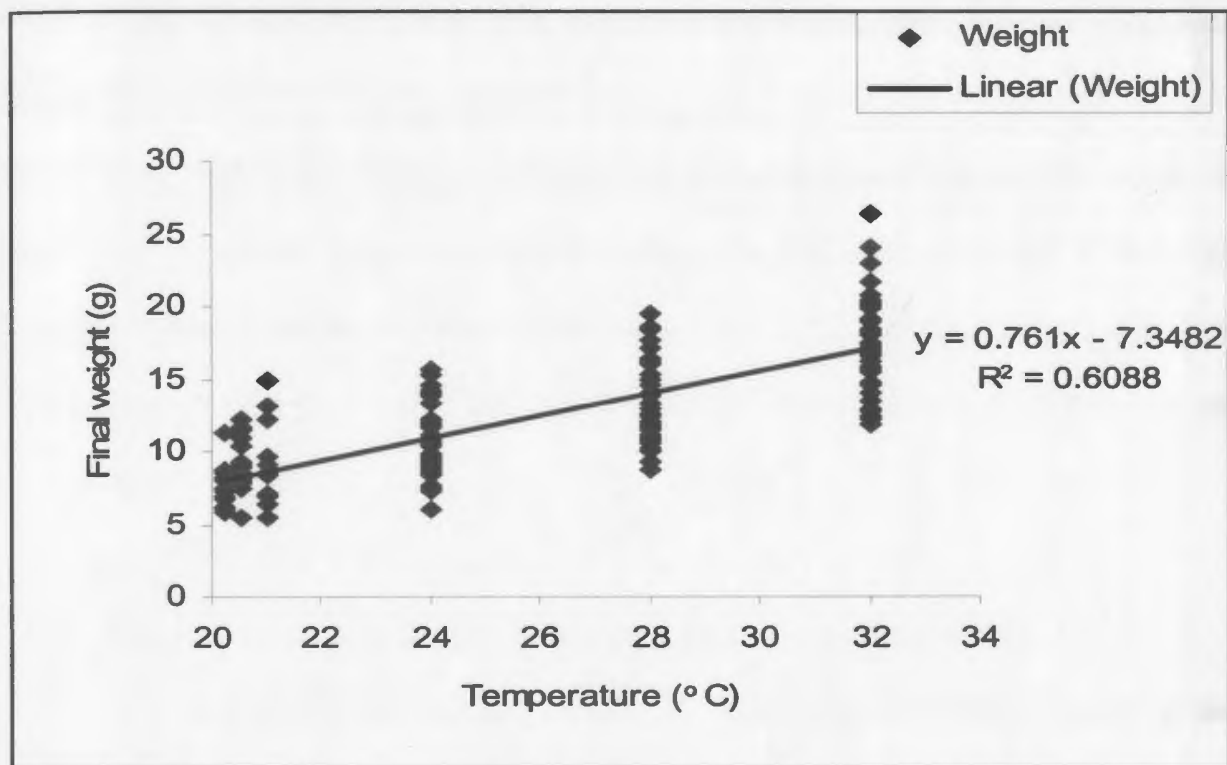


Fig. 4.4. Relationship between temperature and final weight of *Tillapia rendalli* reared in tanks at different temperatures for 70 days. Symbols represent individual fish.

The highest feed conversion ratio (6.3) was found in fish cultured in ambient water temperature. Feed conversion efficiency (FCE) was the highest in fish cultured in the 32° C treatment (59.27%) and lowest in ambient temperature treatment fish (16.83%). This indicates that as temperature increased, the efficiency in utilization of feed also increased (Fig. 4.5).

Temperature levels had an influence on the utilization of protein in the diet and there were significant differences among treatments. Fish cultured at 32° C had higher protein efficiency than those from fish in the rest of the treatments. Thermal unit growth coefficients (TuGC) were significantly different among treatments and followed the same trend (Table 4.5, Fig. 4.5).

4.3.4 Gonado-somatic index (GSI) and gastro-somatic index (GI)

The relationship between the weight of gonads and total body weight, gonado-somatic index (GSI) of *T. rendalli* was significantly influenced by temperature. The highest GSI was found in fish cultured at 32° C followed by fish cultured at 28° C and 24° C, which did not differ significantly ($P < 0.05$) from each other. Significantly lower GSI was found in fish cultured at ambient temperature (Table 4.5).

Gastro-somatic index (GI), which is the relationship between the weight of the gut and total body weight of the fish, was also influenced by temperature. There were significant differences among treatments. The index increased with the increase in temperature from 2.88% in fish cultured at ambient temperature to 4.41% in fish cultured in 32° C water temperatures (Table 4.5).

Table 4.5 Total feed, feed intake, feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER), thermal-unit growth coefficient (TuGC), gonado-somatic index (GSI) and gastro-somatic index (GI) of *Tilapia rendalli* cultured at different temperatures (Mean \pm SE).

Parameter	Treatment			
	Ambient	24° C	28° C	32° C
Total feed (g)	534.54	709.37	785.69	815.97
Feed Intake (% / day)	2.26 \pm 0.05 ^a	2.65 \pm 0.06 ^b	2.54 \pm 0.06 ^b	2.19 \pm 0.05 ^a
FCR	6.30 \pm 1.13 ^d	4.71 \pm 1.10 ^c	2.80 \pm 1.07 ^b	1.78 \pm 1.05 ^a
FCE	16.83 \pm 3.13 ^a	25.42 \pm 2.12 ^b	39.45 \pm 2.58 ^c	59.27 \pm 2.89 ^d
PER	0.56 \pm 0.10 ^a	0.85 \pm 0.07 ^b	1.31 \pm 0.09 ^c	1.98 \pm 0.10 ^d
TuGC	0.05 \pm 0.01 ^a	0.08 \pm 0.01 ^b	0.12 \pm 0.01 ^c	0.16 \pm 0.01 ^d
GSI	0.86 \pm 0.01 ^a	0.89 \pm 0.01 ^{ab}	0.94 \pm 0.01 ^b	1.46 \pm 0.04 ^c
GI	2.88 \pm 0.06 ^a	3.27 \pm 0.06 ^b	3.55 \pm 0.06 ^c	4.41 \pm 0.04 ^d

ANOVA, N=180, Feed intake: $F_{3, 176} = 16.345$, $P < 0.001$; \log_{10} FCR: $F_{3, 176} = 30.668$, $P < 0.001$; FCE: $F_{3, 176} = 47.038$, $P < 0.001$; PER: $F_{3, 176} = 47.038$, $P < 0.001$; TuGC: $F_{3, 176} = 41.34$, $P < 0.001$; N= 36, GSI: $F_{3, 32} = 240.223$, $P < 0.001$; GI: $F_{3, 32} = 128.32$, $P < 0.001$

Note: Values with different superscripts in a row are significantly different (ANOVA, $P < 0.05$); Total feed not tested statistically.

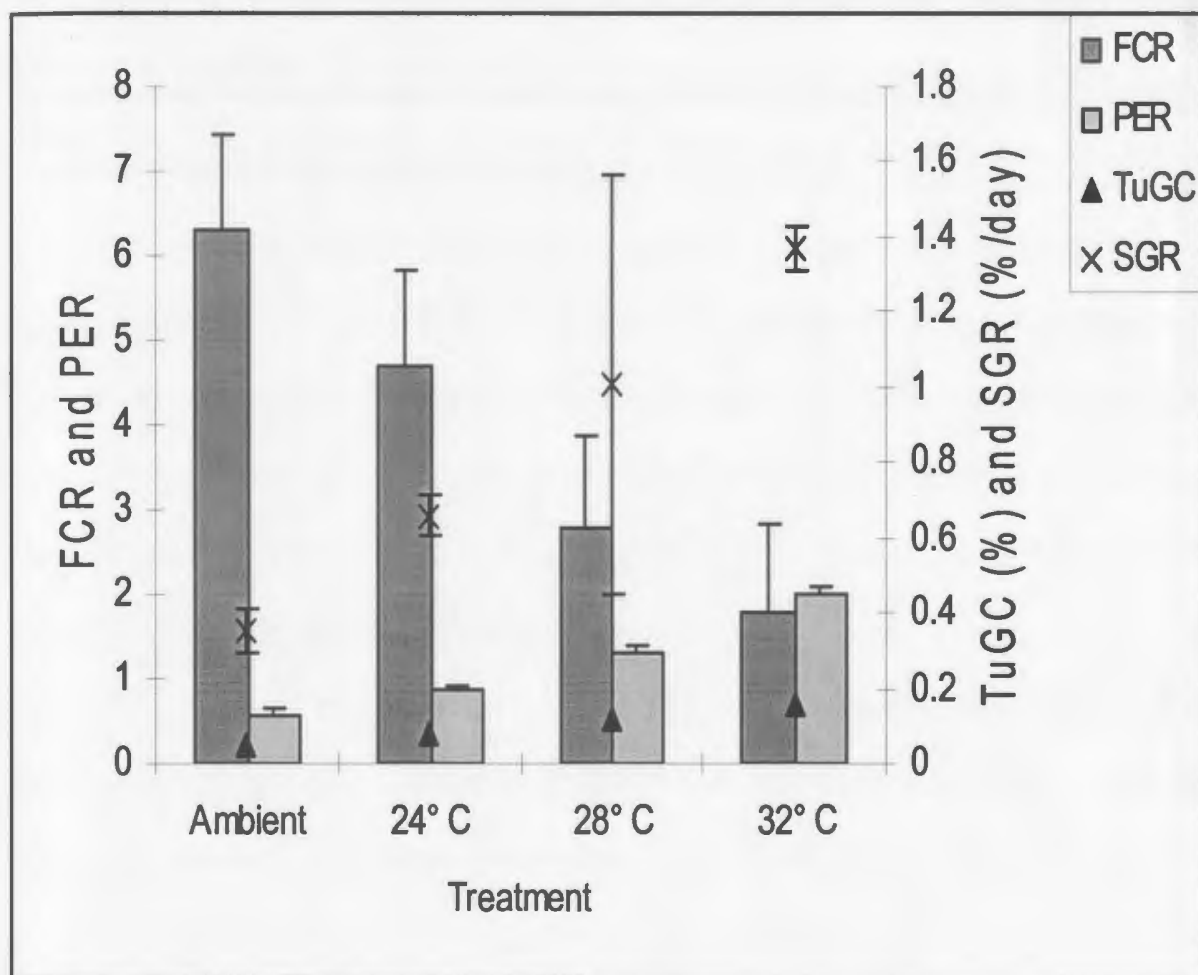


Fig. 4.5. Mean (\pm SE) of feed conversion ratio (FCR), protein efficiency ratio (PER), thermal-unit growth coefficient (TuGC, %) and specific growth rate (SGR, %/day) of *Tilapia rendalli* cultured at different temperatures.

4.3.5 Whole body composition

There were significant ($P < 0.05$) differences in amounts of moisture, ash, crude fat, crude protein and gross energy among the treatments. In comparison with initial composition, moisture levels were significantly different from each other. Moisture decreased from initial levels in all treatments. The lowest amount of moisture was for fish cultured in the 24° C treatment (Table 4.6).

The decrease in ash differed depending on temperature with the larger decrease in fish cultured in ambient temperatures followed by 24° C, 28° C and finally fish in the 32° C treatment, which had the smallest decrease from the initial composition (Table 4.6). Fat content increased in all treatments, but did not depend on temperature, because fish cultured at ambient temperature had significantly higher fat content than fish cultured at 24° C but lower than those cultured in 28° C and 32° C.

Crude protein content was also influenced by temperature. Fish cultured at 32° C had a significantly higher amount of crude protein than the rest. Protein content of fish cultured at ambient temperature decreased from the initial amount of 66.44% to 63.02% but protein content increased in the rest of the treatments. The gross energy content of the fish increased with temperature. The highest amount of energy was in fish cultured at 32° C and the lowest in the ambient temperature treatment (Table 4.6).

4.3.6 Apparent digestibility

Dry matter digestibility coefficients were significantly lower in fish cultured at ambient and 28° C temperatures, and they did not differ significantly between each other.

Table 4.6 Whole body composition (moisture, ash, crude fat, crude protein and gross energy) of *Tilapia rendalli* cultured at different temperatures (Mean \pm SE)

Treatment	Parameter				
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Energy (kJ/g)
Initial	69.27 \pm 0.17 ^c	14.22 \pm 0.15 ^c	9.18 \pm 0.12 ^a	66.44 \pm 0.50 ^b	18.1 \pm 0.08 ^a
Ambient	68.55 \pm 0.17 ^d	9.17 \pm 0.06 ^a	15.34 \pm 0.06 ^c	63.01 \pm 0.15 ^a	26.4 \pm 0.17 ^b
24° C	65.65 \pm 0.06 ^a	9.54 \pm 0.08 ^b	15.03 \pm 0.02 ^b	67.19 \pm 0.09 ^c	30.0 \pm 0.09 ^c
28° C	68.08 \pm 0.06 ^c	10.38 \pm 0.09 ^c	16.64 \pm 0.07 ^d	69.48 \pm 0.26 ^d	31.8 \pm 0.08 ^d
32° C	66.47 \pm 0.47 ^b	11.19 \pm 0.05 ^d	17.25 \pm 0.07 ^e	70.92 \pm 0.12 ^e	33.7 \pm 0.18 ^e

ANOVA, N=38, Moisture: $F_{3, 34} = 165.695$, $P < 0.001$; Ash: $F_{3, 34} = 396.657$, $P < 0.001$; Fat: $F_{3, 34} = 12.865$, $P < 0.001$; Protein: $F_{3, 34} = 295.092$, $P < 0.001$; Gross energy: $F_{3, 34} = 14.05$, $P < 0.001$;

Note: Values with different superscripts in a column are significantly different (ANOVA, $P < 0.05$)

Digestibility coefficients were consistently high in fish cultured at 32° C (Fig 4.6). The digestibility coefficients for crude protein, fat, ash and gross energy increased with temperature, with the lowest digestibility coefficients in fish cultured in ambient temperatures and the highest at 32° C. In general, higher digestibility coefficients were found for protein and gross energy, irrespective of treatment (Fig. 4.6). Appendix 4.2 provides digestibility coefficients.

4.3.7 Water quality parameters

There were significant ($P < 0.05$) differences in pH, conductance, dissolved oxygen, temperature, ammonia and nitrite among treatments (Table 4.7). The pH did not differ significantly at 28° C and 32° C. Conductance significantly increased with temperature, but dissolved oxygen decreased significantly with temperature.

Ammonia and nitrite levels were variable among treatments, with the highest levels recorded at 32° C and the lowest levels at ambient temperatures. However, ammonia and nitrite levels were not significantly different between 24° C and 28° C.

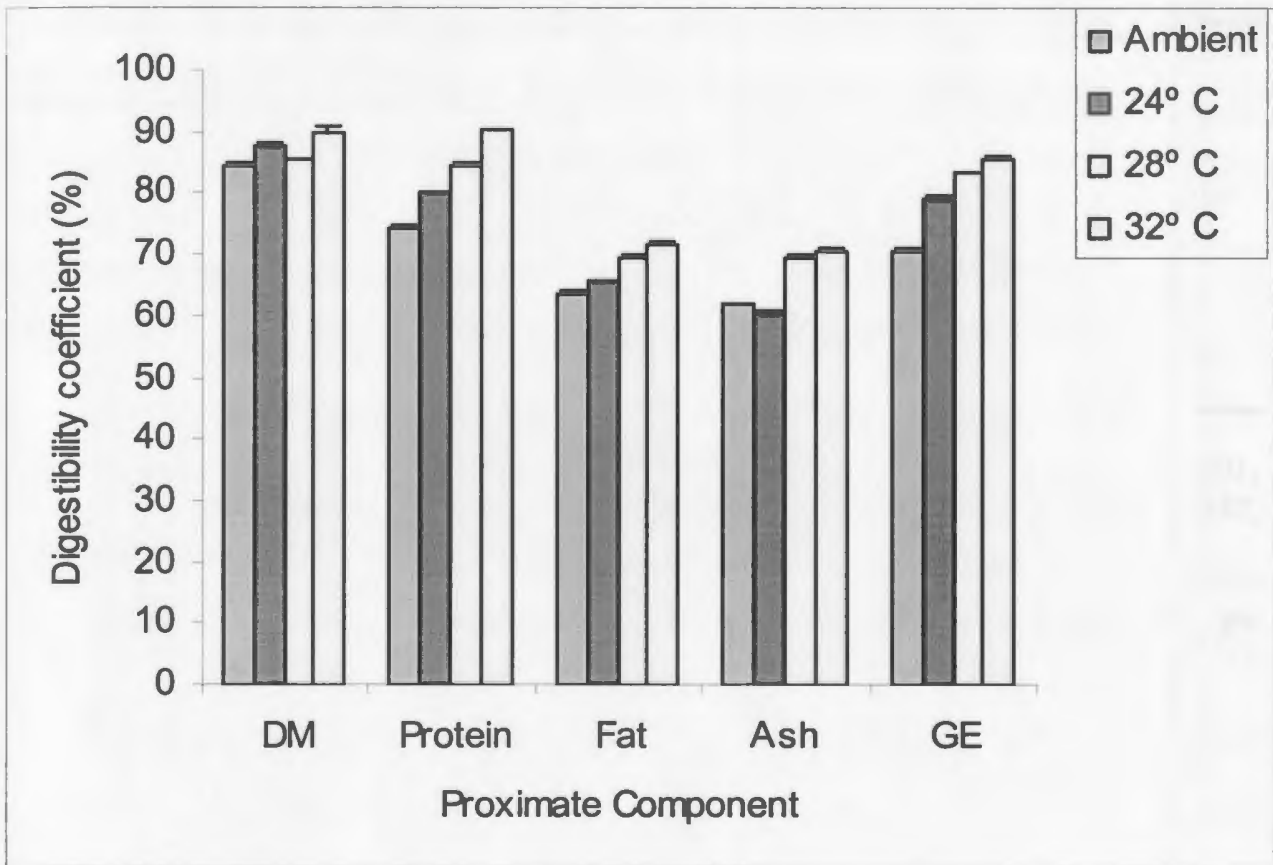


Fig. 4.6. Mean (\pm SE) of the apparent digestibility coefficients of dry matter (DM), protein, fat, ash and gross energy (GE) for *Tilapia rendalli* cultured at different temperatures.

Table 4.7 Water quality parameters for the rearing of *Tilapia rendalli* at different temperatures measured for 70 days (Mean \pm SE).

Parameter	Treatment			
	Ambient	24° C	28° C	32° C
pH	7.57 \pm 0.02 ^c	7.51 \pm 0.01 ^b	7.45 \pm 0.01 ^a	7.47 \pm 0.01 ^a
Conduc. (μ S cm ⁻¹)	685 \pm 7 ^a	733 \pm 7 ^b	805 \pm 9 ^c	882 \pm 20 ^d
Oxygen (mg/L)	5.34 \pm 0.07 ^d	4.40 \pm 0.06 ^c	3.81 \pm 0.05 ^b	3.49 \pm 0.05 ^a
Temperature (° C)	20.5 \pm 0.05 ^a	24.1 \pm 0.01 ^b	28.0 \pm 0.03 ^c	31.8 \pm 0.05 ^d
Ammonia (mg/L)	0.14 \pm 0.02 ^a	0.23 \pm 0.02 ^b	0.28 \pm 0.02 ^b	0.37 \pm 0.02 ^c
Nitrite (mg/L)	0.01 \pm 0.001 ^a	0.02 \pm 0.001 ^b	0.02 \pm 0.001 ^b	0.03 \pm 0.001 ^c

ANOVA, N=840, pH: $F_{3, 836}=47.749$, $P<0.001$; Conductivity: $F_{3, 836}=50.491$, $P<0.001$; Oxygen: $F_{3, 836}=204.958$, $P<0.001$; Temperature: $F_{3, 836}=152.318$, $P<0.001$; N=132, Ammonia: $F_{3, 128}=25.32$, $P<0.001$; Nitrite: $F_{3, 128}=35.513$, $P<0.001$;

Note: Values with different superscripts in a row are significantly different (ANOVA, $P<0.05$)

4.4 Discussion

The growth of *T. rendalli* cultured in tanks was significantly affected by temperature. Temperature affects biochemical reaction rates, which influence energy requirements for repair and maintenance costs, that in turn determine appetite, feeding activity, food consumption, conversion efficiency and growth rates of fish (Liu, 1998). Increase in weight depended on temperature and increased by 170.2% at 32° C and 33.9% in ambient temperature. Faster growth was exhibited at high temperatures than at

ambient treatment (mean: 20.5° C, range: 19.1-21.2° C), which was above the critical temperature (20° C) where tilapia activity and feeding becomes reduced (Chervinski, 1982). Significant differences appeared in the first sampling. This shows that the fish responded quickly to different temperatures. The fish grew from an initial average weight of 6.6 g to 8.65 g, 10.70 g, 13.49 g and 17.37 g at ambient, 24° C, 28° C and 32° C, respectively. This was consistent with the work reported on the feeding and growth of *T. zilli*, with optimum temperature found to be between 28 and 31° C (Platt and Hausser, 1978). In related work, it was found that the growth of rainbow trout also depended on temperature and the total feed requirement was proportional to the rate of weight gain (Watanabe, 1988). The present results showed that the fastest growth of juvenile *T. rendalli* was obtained at 32° C. Below this temperature, the ambient temperature probably represented the minimal thermal limit for growth. Tilapia in general are considered to show best growth rates between 26 and 28° C (Maruyama, 1983) and beyond (Caulton, 1982; Bhikajee and Gobin, 1997) under laboratory conditions, but their performance depends on acclimation as well-acclimated fish perform effectively (Rajaguru and Ramachandran, 2001; Aguiar *et al.*, 2002; Rajaguru, 2002).

The trend in growth of the fish was reflected in individual weight gains and specific growth rates (SGR) among treatments. The weight gains and SGRs of fish in this study increased with temperature and this agrees with Bhikajee and Gobin (1997), who reported a similar trend in red tilapia hybrid raised in similar temperatures. They reported higher SGRs (1.7-3.2) than those in the present experiment (0.35-1.37). This could be due to differences in the diets (all-plant vs. commercial) and the growth characteristics of

these species, with red tilapia having higher growth than *Tilapia rendalli*. In bluefish, growth was affected by temperature and fish size, with medium and large fish continuing to increase in weight at higher temperatures, whereas growth of small fish increased above 24° C (Buckel, *et al.*, 1995). However, the medium sizes used in their experiment are comparable to the initial weights used in this experiment.

Feed utilization in this study also depended on temperature. Fish cultured at high temperatures consumed high total amounts of feed and those in ambient water temperatures consumed the lowest total amount of feed. The total amount of feed consumed increased with temperature and this is in accordance with feed consumption in bluefish (Buckel *et al.*, 1995) and *Esox licius* (Salam and Davies, 1994) under laboratory conditions. The increase in feed consumption could have resulted in high oxygen consumption in fish held at high temperatures. Increased feed intake leads to elevated oxygen consumption (Jobling, 1981; Cui and Wootton, 1988; Ross, 2000) though this depends on the species, ration size and temperature (Jobling, 1981). The diet used in the present experiment had a high protein content (30%), which could have resulted in the fish consuming high levels of oxygen as consumption increases with protein in tilapia (Ross, 2000). The daily ingestion of *Tilapia rendalli* juveniles exposed to temperatures ranging from 18 to 34° C increased with temperature but decreased dramatically beyond 34° C (Caulton, 1982). The overall feed intake (%/day) of fish during the present experiment was not temperature dependent since significantly lower feed intake was found in fish at the highest and ambient temperatures. This may be due to the fact that the feed was controlled according to body weight (3%) and was not *ad libitum*. Despite that,

the fish at 32° C grew faster than the rest of the fish in other treatments, as a relatively low amount of feed was consumed in relation to body weight, indicating high feed efficiency (Salam and Davies, 1994). The low feed intake at ambient temperatures could be reflecting actual reduced feed intake at low temperatures (Blakely and Hrusa, 1989) as this corresponds to the lowest amount of feed consumed and the lowest growth rate. Fish cultured at 32° C had better FCR, FCE and PER than the fish in the rest of the treatments and the trend compares well with work reported with red tilapia hybrids (Bhikajee and Gobin, 1997). Lower FCRs obtained in this experiment, which utilized an all-plant diet, are comparable to those obtained in fish fed diets formulated from animal protein for tilapia and other fishes. The feed efficiency increase in fish raised at higher temperatures over ambient temperatures could be due to changes in metabolic rate, which is more elevated in tropical fish than temperate fish (Pauly, 1998). This may have improved oxygen intake (Brett and Groves, 1979; Lyytikainen and Jobling, 1998; Ross, 2000), which could facilitate increased digestion and assimilation of feed during metabolism, and would eventually influence their growth (Bowen, 1982; Mwangangi and Mutungi, 1994; Bhikajee and Gobin, 1997; Pauly, 1998).

Thermal unit growth coefficient (TuGC) indicated that temperature had an influence on growth. TuGC increased with temperature. This confirmed the trend of SGRs, which were also significantly higher at high temperatures. Many species of fish thermo-regulate differently, preferring temperatures that are close to their optimum for growth (Hill and Magnuson, 1990; Wildhaber and Crowder, 1990; Mwangangi and Mutungi, 1994). As a result the preference of the fish tends to coincide with the optimal

temperature for growth (Hill and Magnuson, 1990; Gadomski and Caddell, 1991). Therefore, it is important to keep the water temperature at optimum levels to maintain maximum feed intake and optimal metabolic processes (Watanabe, 1988).

The gonado-somatic index (GSI) was significantly higher at highest temperatures. This agrees with the work reported earlier that low temperatures (15-22° C) inhibit reproduction in tilapia (Jalabert and Zohar, 1982). Fish in the present experiment exhibited high indexes in high water temperatures, though there was no significant difference between fish in the 24° C and 28° C treatments. Fish directed energy to the growth of their gonads during this time in the high temperatures. Although gonad sizes were small due to the small size of the fish at the termination of the experiment, the growth of the gonads was seen to increase with temperature. However GSI, peaked at lower temperatures (20° C) in juvenile sea horses, *Hippocampus whitei* (Wong and Benzie, 2003). This may be due to differences in fish species, feed used and acclimation. Gastro-somatic index (GI) was also significantly higher in fish at 32° C and lowest at ambient temperature. This could be because the fish cultured at high temperatures had a lot of fat in their intestines, upon dissection compared to those in lower temperatures.

The whole body proximate composition of the fish reflected the influence of temperature. There was a decrease in moisture and ash contents among treatments. The moisture content did not depend on temperature, but the decrease in ash content of the fish depended on temperature and the decrease in these two components is common in similar feeding experiments. There was an increase in fat levels in fish at high temperatures and this was reflected in an elevated energy content of the fish with

temperature, which is similar to results in other studies (Imsland *et al.*, 2001). Protein levels increased in all treatments except ambient temperature. This could be due to the fact that the fish in ambient temperatures lost weight, and therefore did not utilize the protein for growth but used it for energy (Caulton, 1982).

Apparent digestibility coefficients for various proximate components of the diets were variable but increased with temperature, except dry matter and ash, which did not depend on temperature. Dry matter digestibility was significantly lower at 28° C (85.30%) and ambient temperatures (84.73%), while high dry matter digestibilities were exhibited at 24° C (86.76 %) and 32° C (89.83) water temperatures. Protein, fat and energy digestibility coefficients increased with temperature. This could be due to an increase in activity of the enzymes responsible for digestion during metabolism, which increases with temperature (Caulton, 1982). At 32° C, protein had the highest digestibility coefficient and ash the lowest. However, at ambient temperatures, dry matter had the highest digestibility coefficient and the ash lowest. An increase in gastric pH has been noted in channel catfish with temperature but is more pronounced when feeding is high (Newton and Burtle, 1995). The increased digestibility of protein, fat and energy, which corresponded to an increase in feed intake by the fish with temperature increase could be due to increases in gastric pH that would be responsible for the effective performance of digestive enzymes (Arlinghaus *et al.*, 2003).

Survival rate of the fish was high considering the static system in which the fish were cultured. The daily water change improved the water quality in the tanks. Though there were fluctuations in dissolved oxygen (DO), the level of DO was above 3 mg/L in

all treatments, which is considered ideal for warm water fishes (Buttner *et al.*, 1993). This was still above 2.5 and 1 mg/L DO where *O. noloticus* showed signs of stress (decrease in activity accompanied by body coloration and erection of dorsal fin) in similar systems (Ross, 2000). The pH of the water was within the limits for the growth of many tilapias (Boyd and Tucker, 1998; Ross, 2000). The mean pH was about 7.5 across the treatments, though the high temperatures had significantly lower pH than the two lower temperatures. Ammonia and nitrite levels were elevated at high temperature compared to ambient temperatures and this is in accordance to some physiological studies done with Arctic charr under different temperatures (Lyytikaines and Jobling, 1998). Ammonia remained within acceptable levels for other tilapia (Chervinski, 1982).

4.5 Conclusion

Temperature had a significant effect on the growth of *T. rendalli* raised at different water temperatures under laboratory condition. Growth increased with temperature achieving the highest SGR at 32° C and lowest at ambient temperatures.

Feed utilization, as measured by FCR and FCE, followed the trend of growth. This showed that *T. rendalli* utilized the feed by achieving lower feed conversion at highest temperatures (32° C), which coincided with the high conversion efficiency. The utilization of feed improved with temperature from ambient temperatures to 32° C. This was also reflected in the total amount of feed consumed by the fish during the experimental period, which was temperature dependent.

Although the diets were formulated from all-plant sources (maize bran, rice bran, cotton seed cake and soybean meal) there was evidence of high digestibility with an increase in temperature. However, dry matter and ash digestibility had coefficients, which did not depend on temperature. The survival of the fish was not affected by temperature, as the survival was 100%. The results indicated that 32° C might be the optimal temperature for *T. rendalli*.

Chapter 5

EFFECT OF SALINITY ON GROWTH, FEED UTILIZATION AND SURVIVAL OF *TILAPIA RENDALLI* IN AQUARIA

5.1 Introduction

Salinity influences the growth performance of many fish, and have been studied in several species of fish in ponds, tanks, raceways and cages (Fineman-Kalio, 1988; Al-Ahmad *et al.*, 1988; Hopkins *et al.*, 1989; Cruz *et al.*, 1990; Watanabe *et al.*, 1990). However, these studies have been limited to *O. aureus*, *O. niloticus*, *O. spilurus*, *O. mossambicus* and Florida red tilapia that are referred to as saline tolerant. Little work has been reported on salinity effects on growth and feed utilization of other potential saline tolerant cultured fish species (Watanabe *et al.*, 1988; Likongwe, 1995; Likongwe, 2002), although it is believed that all freshwater tilapia are tolerant to brackish water (Chervinski, 1982; Stickney, 1986; Watanabe, 1991; Suresh and Kwei Lin, 1992; Popma and Masser, 1999). Sometimes the relationship between salinity and growth may be complex and not readily predicted (Iwama, 1996; Elghohashy, 2001). The combined effects of temperature and salinity had a significant influence on growth of *O. niloticus* under culture. Watanabe *et al.* (1997) indicated that salinity effects vary ontogenetically in tilapia with tolerance improving markedly around 40 days post-hatching, which is the recommended age for acclimating fry to seawater.

The effects of salinity on growth in tilapias are complex and can be modified by the interactive effects of both non-osmoregulatory (territorial aggression) and osmoregulatory factors on metabolism. Under controlled photoperiod (12 L: 12 D) and temperature at 28° C, growth of juvenile, sex reversed, male Florida red tilapia was higher in brackishwater ($\geq 10\text{‰}$) and seawaters (36‰) than in 1‰ freshwater (Watanabe *et al.*, 1997). Pond culture of tilapia and red tilapia, in saline waters (10‰) was also

attempted in Thailand at the Asian Institute of Technology (AIT) (Yi *et al.*, 2002). They found that fish growth and average feeding rate increased, but feed conversion ratio (FCR) and net economic return decreased with increasing percentages of satiation feeding levels from 25 to 100%.

Pond water salinity changes during periods of high precipitation or evaporation. This is pronounced in the tropics where the climate is characterised by wet and dry seasons and the change can be significant (Suresh and Kwei Lin, 1992; Boyd and Tucker, 1998). In Western Australia, salinity in farm ponds can fluctuate from less than 0.5‰ in the wet season to over 3‰ in dry season (Boyd and Tucker, 1998). Such changes may influence the growth of fish. Malawi is not an exception. It has areas where soils are too saline to support crop husbandry, but brackish water may be used for aquaculture if salinity tolerant species are used (Likongwe, 2002). In Malawi, preliminary studies have recently been done on several Malawian tilapia including *T. rendalli* (Likongwe, 2002). Although farmers in these areas raise fish, the potential for increasing production utilizing salinity tolerant fish remains untapped. Therefore, the following experiment was carried out under laboratory condition to investigate the potential of raising tilapia in brackish water. The experiment investigated the effect of salinity levels on the growth, feed utilization and survival of *Tilapia rendalli*.

5.2 Materials and methods

5.2.1 Experimental facilities and set-up

The experiment was conducted in the laboratory at the Aquaculture experimental farm of the Department of Aquaculture and Fisheries Science at Bunda College of Agriculture. Twelve 50 L glass tanks were set up with a bio-filter (Matsumoto) situated in the center of each tank. Four salinity level treatments (freshwater ($<0.5\text{‰}$), 5‰ , 10‰ and 15‰) were assigned to the tanks in a completely randomised design (CRD), with three replicates for each treatment. Levels of salinity were reached by increasing the salt (sodium chloride) added to the freshwater by 2‰ every day across the treatments until the desired levels were reached. A salinity probe (Horiba Ltd, Japan) was used to monitor salinity in the water every day. Water with different salinity levels was prepared in advance in separate tanks for replacement every day. The experiment was conducted under ambient photoperiod.

A preliminary experiment was conducted to test salinity levels of freshwater 10‰ , 15‰ , and 20‰ . After two weeks it was discovered that the fish died ($>50\%$) within two weeks at 20‰ . This agrees with earlier studies with *T. rendalli* (Whitfield and Blaber, 1976; Baralin and Hutton, 1979; Likongwe, 2002). The design was reconsidered and based on the preliminary studies, the experiment was as outlined above.

5.2.2 Experimental animals and stocking

The fish used during this experiment initially averaged 3.94 ± 0.44 g and 5.83 ± 0.25 cm. The acclimation, stocking and sampling procedures were as described in section 4.2.2 (Chapter 4).

5.2.3 Feeds and feeding

5.2.3.1 Acquisition of the feed ingredients used in the experiment

The same ingredients used in the temperature experiment described in section 4.2.3.1 (Chapter 4) were used in this experiment.

5.2.3.2 Proximate analysis of feed ingredients

The proximate analysis of the ingredients and formulated diets used in this experiment are as presented in section 3.2.2 (Chapter 3) (Tables 4.1 and 4.3).

5.2.3.3 Fish feeding

The diet came from the same batch that was prepared in the previous experiment (Chapter 4) and was stored frozen. Feeding was done twice a day at 3 % ($2 \times 1.5\%$) body weight. All feeding and tank maintenance protocols were the same as reported in section 4.2.3.6 (Chapter 4).

5.2.4 Fish weighing and data collection

5.2.4.1 Fish weighing

The protocol used was the same as described in section 4.2.4.1 (Chapter 4).

5.2.4.2 Fish growth performance

Data collection was the same as described in section 4.2.4.2 (Chapter 4).

5.2.4.3 Growth performance Index

Indices showing the performance of the fish such as gonado-somatic index (GSI) (%), gastro-somatic index (GI) (%) and thermal unit growth coefficient (TuGC) were calculated using formulae as outlined in section 4.2.4.3 (Chapter 4). Fulton's condition factor was calculated using the formula outlined in section 2.2.5 (Chapter 2).

5.2.4.4 Feed and protein utilization

The feed utilization was calculated as described in section 4.2.4.4 (Chapter 4).

5.2.4.5 Analysis of fish body composition

The protocol used was the same as described in section 3.2.10 (Chapter 3).

5.2.5 Digestibility determination

The protocols were as described in section 4.2.5 (Chapter 4). This trial was conducted for 14 days after a 70-day growing period. The digestibility coefficients for nutrients and energy were calculated using the formula as outlined in NRC (1993).

5.2.6 Water quality monitoring

The protocol used was the same as described in section 4.2.7 (Chapter 4).

5.2.7 Data collection and statistical analysis

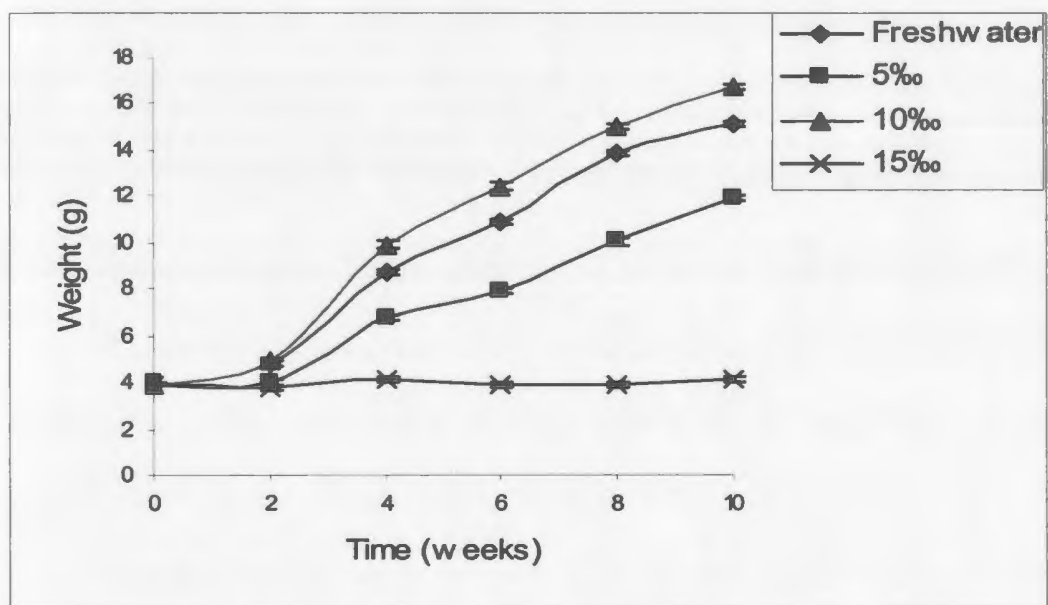
The data were collected as in Chapter 4. The data were subjected to non-parametric analysis using Kruskal-Wallis test for mean weight, weight gains, feed intake, and feed conversion and protein efficiency ratios after the data were found not to meet the assumptions for analysis of variance (ANOVA). Individual means of all parameters analysed in this experiment were compared at 5% alpha level of significance for differences due to treatments and ranked by comparing the group. One-way ANOVA was performed on fish body composition parameters, digestibility and water quality parameters. In this case individual means were compared at 5% alpha level of significance and Duncan's multiple range tests were used to test for differences due to treatments. Regression analysis was performed on the final weight and salinity to assess their relationship. The data are presented as means \pm SE of three replicate groups. All statistical analyses were carried out using SPSS 10.0 (SPSS Inc., 1999).

5.3 Results

5.3.1 Body weight

The initial average body weights of the fish were not significantly different ($P > 0.05$). There were significant differences in average weight with time and final average weight did not depend on salinity. Significant ($P < 0.05$) differences started appearing just two weeks into the experiment in the first sampling (Fig 5.1). In two weeks the average weights of the fish in freshwater and in the 10‰ treatment were significantly higher than those in 5‰ and 15‰ levels of salinity (Fig. 5.1). The final average weights of fish cultured in 15‰ were lowest (4.09 g) with the highest in the 10‰ salinity level (16.73 g). The average weight in the freshwater treatment was significantly higher than those cultured in 5‰ and the trend was the same with average total lengths over the experimental period (Fig. 5.1). The growth of fish cultured in 15‰ salinity was almost constant, as compared to the rest of the treatments, but the fish cultured in 10‰ salinity level demonstrated high growth. The effect of salinity on growth was generally negative as growth decreased with salinity. The polynomial regression analysis indicated a significant ($F_{1,178} = 112$, $P < 0.001$, $R^2 = 0.6262$) relationship between the final weight and salinity with the following equation: $\text{weight} = -0.0903x^2 + 0.8047x + 13.93$ (Fig 5.2, Appendix 5.1).

a)



b)

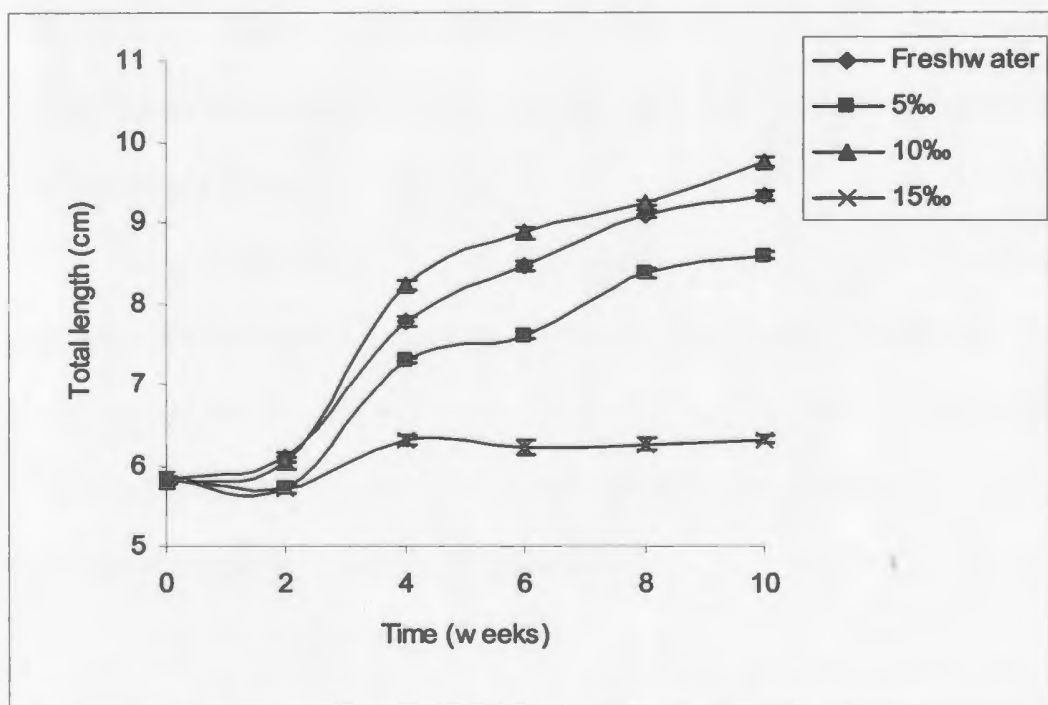


Fig. 5.1. Mean (\pm SE) of weight (a) and total length (b) of *Tilapia rendalli* cultured under different salinities for 70 days.

5.3.2 Growth characteristics, condition and survival of fish

There were significant ($P < 0.05$) differences in total weight gains of the fish among treatments. The highest weight gain was obtained in fish cultured in 10‰ (12.77 g) and the lowest in those cultured in 15‰ (0.12 g). The weight gains of fish in freshwater were significantly higher than those cultured in 5‰ (Table 5.1).

The specific growth rates (SGR) of fish were also highest in fish cultured in 10 ‰ (2.06 %/day) followed by those in fresh water (1.93 %/day), 5‰ (1.58 %/day) and lastly in 15‰ (0.04 %/day). The growth rates did not depend on salinity.

The percent increase in weight of the fish at the end of the experiment was highest in fish cultured at 10 ‰ (327%) followed by freshwater (289%), 5‰ (205%) and lastly 15‰ (4.8%). The growth of fish indicated that the fish in 10‰ salinity level grew 321.9% better than those cultured in 15‰ salinity level. This growth trend was also true for daily weight gains (Table 5.1).

The condition of the fish was significantly different among treatments. Fish cultured in the highest salinity level (15‰) had significantly lower condition than the fish in the rest of the treatments, which did not differ significantly from each other (Table 5.1). The survival of the fish (Fig. 5.3) decreased with salinity from 100% to 66.7% and were significantly different across treatments.

5.3.3 Feed utilization

Total feed intake was variable among treatments and was not salinity dependent. The highest amount of total feed was recorded for freshwater fish followed by 10‰, 5‰

and the lowest intake of feed was recorded for fish in 15‰ salinity (Table 5.2). The percent intake of feed per day differed significantly among treatments with the fish cultured in freshwater and 15‰ salinity level having significantly ($P < 0.05$) lower intake of feed than 5‰ and 10‰ salinity levels but these levels, did not differ significantly from each other (Table 5.2).

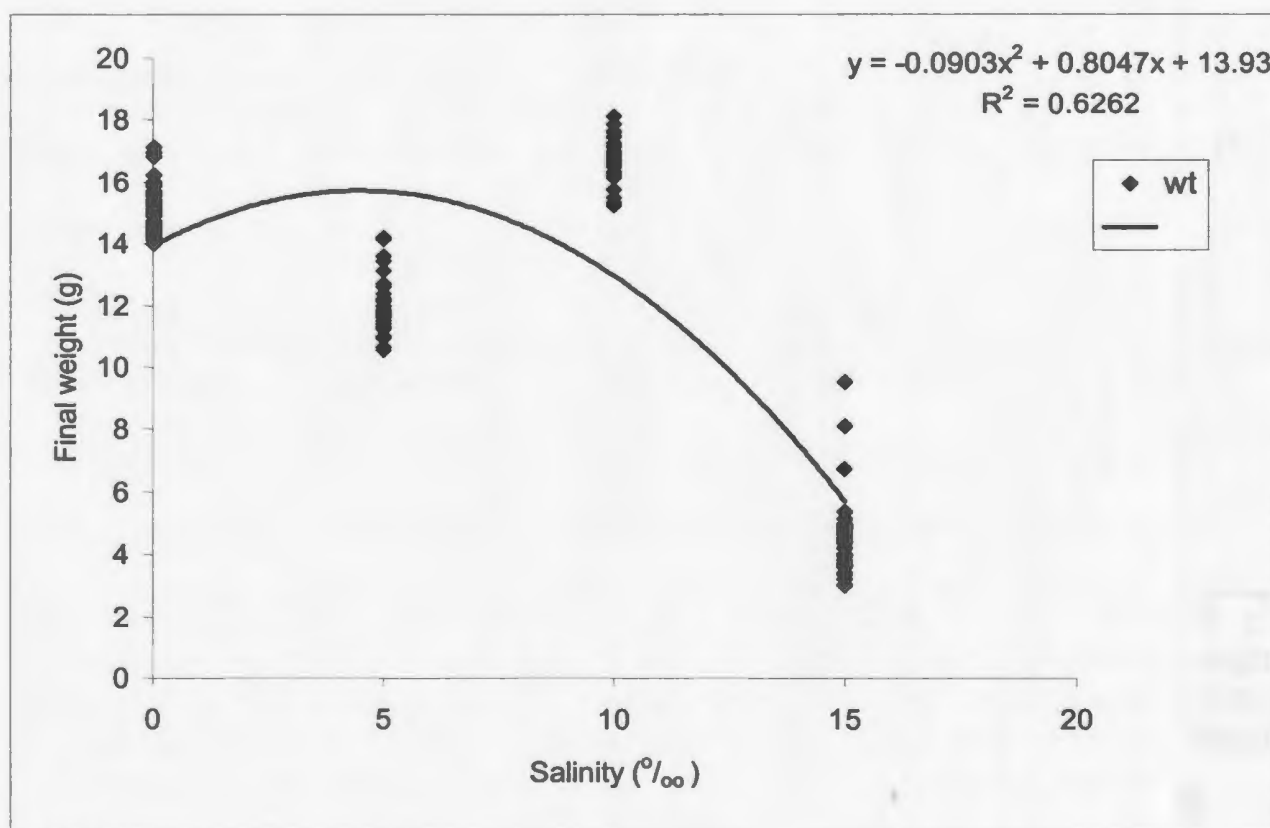


Fig. 5.2. Relationship between salinity and final weight of *Tillapia rendalli* cultured at different salinities for 70 days. Symbols represent individual fish.

Table 5.1 Initial weight, final weight, weight gain, weight gain per day, weight increase, specific growth rate (SGR), initial condition, final condition and survival of *Tilapia rendalli* cultured at different salinities (Mean \pm SE).

Parameter	Treatment			
	Freshwater	5‰	10‰	15‰
Initial wt (g)	3.95 \pm 0.07	3.96 \pm 0.06	3.92 \pm 0.06	3.94 \pm 0.07
Final weight (g)	15.14 \pm 0.12 ^c	11.93 \pm 0.13 ^b	16.73 \pm 0.10 ^d	4.09 \pm 0.10 ^a
Weight gain (g)	11.19 \pm 0.15 ^c	7.98 \pm 0.14 ^b	12.77 \pm 0.12 ^d	0.12 \pm 0.14 ^a
Weight gain/day	0.16 \pm 0.00 ^c	0.11 \pm 0.00 ^b	0.18 \pm 0.00 ^d	0.002 \pm 0.002 ^a
Weight increase (%)	289 \pm 8 ^c	205 \pm 5 ^b	327 \pm 7 ^d	4.8 \pm 3.7 ^a
SGR (%/day)	1.93 \pm 0.03 ^c	1.58 \pm 0.03 ^b	2.06 \pm 0.02 ^d	0.04 \pm 0.05 ^a
Initial condition	1.99 \pm 0.03	2.04 \pm 0.06	2.00 \pm 0.03	1.97 \pm 0.02
Final condition	1.88 \pm 0.05 ^b	1.88 \pm 0.02 ^b	1.81 \pm 0.02 ^b	1.61 \pm 0.02 ^a
Survival (%) ¹	100.0 \pm 0.0 ^c	95.6 \pm 2.2 ^{bc}	88.9 \pm 2.2 ^b	66.7 \pm 3.8 ^a

Kruskal-Wallis: Initial wt: N= 180, χ^2 =0.175, df=3, P =0.98; Final wt: N= 159, χ^2 =142.420, df=3, P < 0.001; Weight gain: N= 159, χ^2 =139.658, df=3, P < 0.001; Weight gain/day: χ^2 =139.658, df=3, P < 0.001; Weight incr: χ^2 =122.995, df=3, P < 0.001; SGR: χ^2 =122.995, df=3, P < 0.001; Initial condition: χ^2 =0.206, df=3, P =0.977; Final condition: χ^2 =54.410, df=3, P < 0.001. Survival, ANOVA, $F_{3,8}$ =35.483, P <0.001

Note: Values with different superscripts in a row are significantly different (Kruskal-Wallis, P < 0.05); ¹ (ANOVA, P < 0.05)

Significantly higher FCRs were calculated for fish cultured in the 15‰ salinity (14.91) and lowest in 10‰ salinity. FCRs for freshwater, 5‰ and 10‰ treatments did not differ significantly from each other (Fig. 5.4). Feed conversion efficiency was also the lowest (13.30 %) at the highest salinity and highest for fish cultured in 10‰ (76.37%). This did not differ significantly with those in freshwater and 5‰ salinity. The protein efficiency ratio (PER) was also lowest for fish cultured in 15‰ salinity (0.44) and highest in freshwater (3.36). Freshwater PER did not differ significantly with those fish in the 5‰ and 10‰ salinity. The relationships between FCR, PER, TuGC and SGR showed a similar pattern with growth of fish (Fig. 5.4).

5.3.4 Gonado-somatic index (GSI) and gastro-somatic index (GI)

Gonado-somatic index (GSI) and gastro-somatic index (GI) were both significantly ($P < 0.05$) different due to treatment. Fish in 15‰ salinity had the lowest GSI and GI while the highest were recorded in fish cultured in 10‰ salinity level (Table 5.2). The indices in freshwater and 5‰ salinity did not differ significantly from each other. The TuGC, which is a measure of growth in relation to temperature over time, differed significantly among treatments with the lowest in fish cultured in 5‰ and highest in 10‰. Those in 15‰ and freshwater did not differ significantly. This indicates that the growth of the fish in relation to gonads, gastro intestines and temperature did not depend on salinity (Table 5.2).

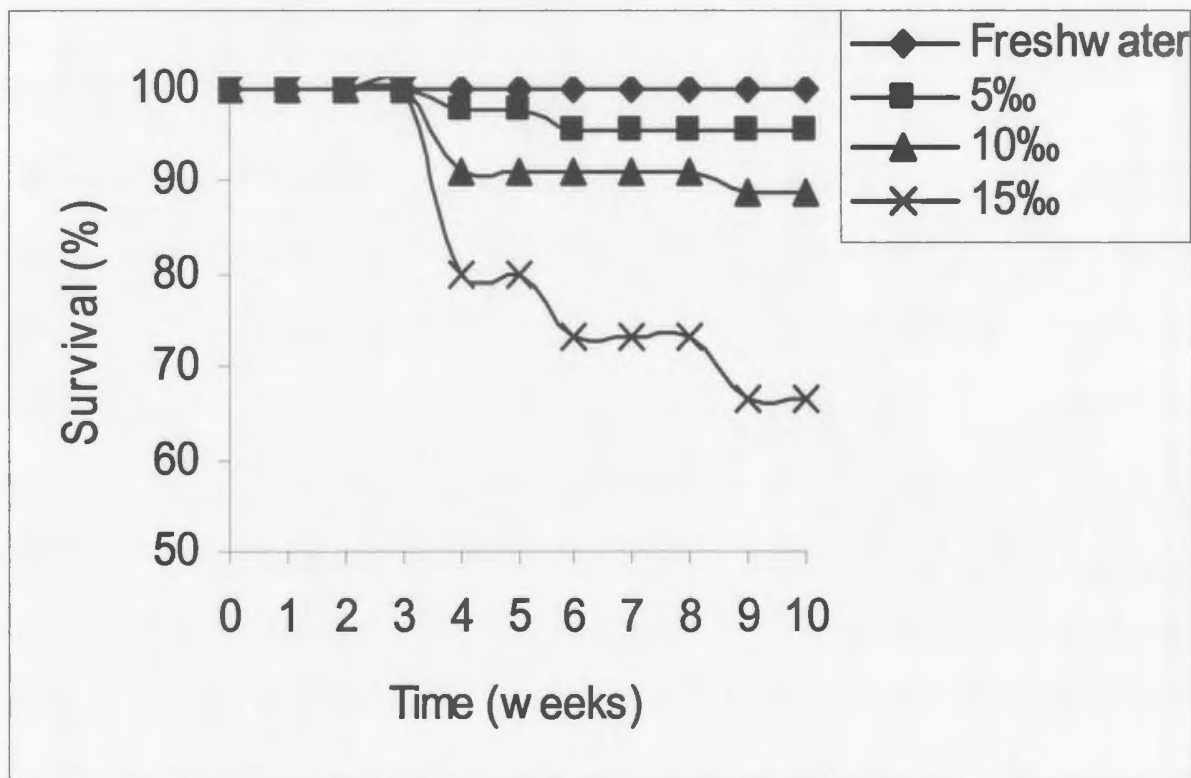


Fig. 5.3. Survival (%) of *Tilapia rendalli* cultured at different salinities over the experimental period of 70 days.

Table 5.2 Total feed, feed intake, feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER), thermal-unit growth coefficient (TuGC), gonadosomatic index (GSI) and gatroscopic index (GI) of *Tilapia rendalli* cultured at different salinities (Mean \pm SE).

Parameter	Treatment			
	Freshwater	5‰	10‰	15‰
Total feed (g)	711.81	490.81	652.99	182.37
Feed In. (%/day)	2.37 \pm 0.02 ^a	2.06 \pm 0.02 ^b	2.32 \pm 0.05 ^b	2.07 \pm 0.05 ^a
FCR	1.42 \pm 0.02 ^a	1.45 \pm 0.02 ^a	1.36 \pm 0.06 ^a	14.91 \pm 15.1 ^b
FCE	70.74 \pm 0.95 ^b	69.85 \pm 1.15 ^b	76.37 \pm 1.53 ^b	13.30 \pm 5.10 ^a
PER	3.36 \pm 0.03 ^b	2.32 \pm 0.04 ^b	2.55 \pm 0.05 ^b	0.44 \pm 0.17 ^a
TuGC	0.25 \pm 0.00 ^b	0.18 \pm 0.00 ^a	0.28 \pm 0.01 ^c	0.24 \pm 0.01 ^b
GSI ¹	0.89 \pm 0.01 ^b	0.91 \pm 0.01 ^b	1.39 \pm 0.02 ^c	0.46 \pm 0.02 ^a
GI ²	3.08 \pm 0.06 ^b	2.99 \pm 0.02 ^b	3.84 \pm 0.04 ^c	2.05 \pm 0.04 ^a

Kruskal-Wallis: N= 159. Feed intake: χ^2 =85.119, df=3, P < 0.001; FCR: χ^2 =25.984, df=3, P < 0.001; FCE: χ^2 =98.450, df=3, P < 0.001; PER: χ^2 =98.450, df=3, P < 0.001; TuGC: χ^2 =90.962, df=3, P < 0.001; GSI: ANOVA, N= 36, $F_{3,32}$ =50.296, P < 0.001; GI: $F_{3,32}$ =31.257, P < 0.001.

Note: Values with different superscripts in a row are significantly different (Kruskal-Wallis, P < 0.05); ^{1,2} (ANOVA, P < 0.05); Total feed not statistically tested.

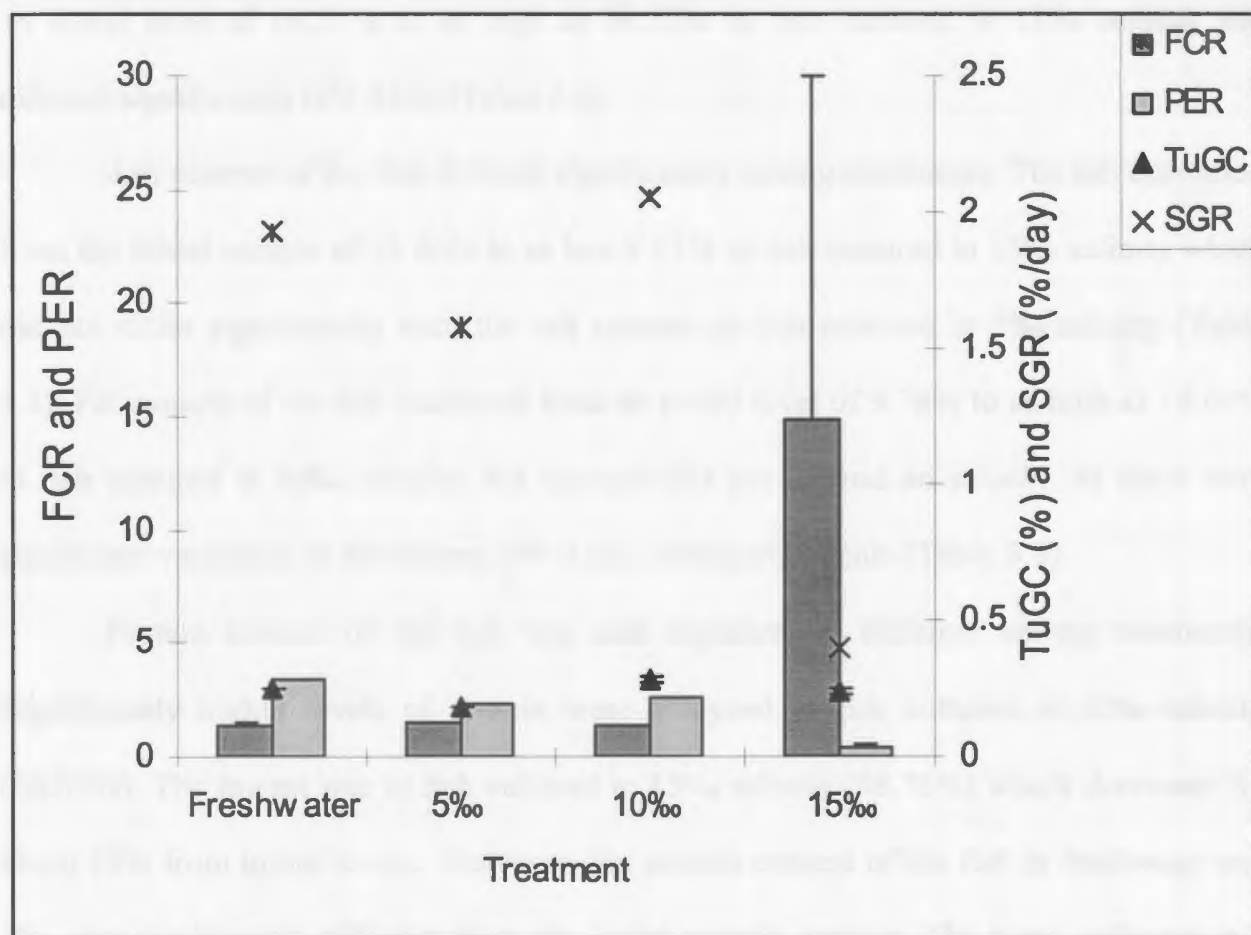


Fig. 5.4. Mean (\pm SE) of feed conversion ratio (FCR), protein efficiency ratio (PER), thermal-unit growth coefficient (TuGC, %) and specific growth rate (SGR, %/day) of *Tilapia rendalli* cultured at different salinities.

5.3.5 Whole body composition

The whole body composition of the fish at the end of the experiment was compared to the initial composition of the fish. Moisture increased in all treatments from an initial level of 64.57% to as high as 69.33% in fish cultured in 15‰ salinity and differed significantly ($P < 0.05$) (Table 5.3).

Ash content of the fish differed significantly among treatments. The ash decreased from the initial sample of 13.60% to as low 9.11% in fish cultured in 15‰ salinity which did not differ significantly with the ash content of fish cultured in 5‰ salinity (Table 5.3). Fat content of the fish increased from an initial level of 9.78% to as high as 16.69% in fish cultured in 10‰ salinity. Fat increase did not depend on salinity, as there were significant variations in fat content ($P < 0.05$) among treatments (Table 5.3).

Protein content of the fish was also significantly different among treatments. Significantly higher levels of protein were analyzed in fish cultured at 10‰ salinity (68.67%). The lowest was in fish cultured in 15‰ salinity (58.75%) which decreased by about 12% from initial levels. However, the protein content of the fish in freshwater and 5‰ was significantly different from the initial protein content. The trend indicated that the fish maintained or increased their protein level during the experimental period except for those fish cultured in 15‰ salinity (Table 5.3).

Gross energy content increased across the treatments and their levels were significantly different from each other. Gross energy increased from an initial level of 18.7 kJ/g to as high as 31.8 kJ/g in fish cultured at 10‰ and the increase did not depend on the salinity level of the water.

Table 5.3 Whole body composition (moisture, ash, crude fat, crude protein and gross energy) of tilapia cultured at different salinities (Mean \pm SE).

Treatment	Parameter				
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Energy (kJ/g)
Initial	64.57 \pm 0.25 ^a	13.60 \pm 0.25 ^c	9.78 \pm 0.16 ^a	67.12 \pm 0.08 ^b	18.7 \pm 0.24 ^a
Freshwater	67.68 \pm 0.07 ^b	9.88 \pm 0.05 ^b	15.52 \pm 0.04 ^c	67.00 \pm 0.13 ^b	26.9 \pm 0.09 ^d
5‰	68.58 \pm 0.10 ^d	8.85 \pm 0.05 ^a	15.22 \pm 0.05 ^d	66.65 \pm 0.09 ^b	26.1 \pm 0.07 ^c
10‰	68.05 \pm 0.07 ^c	9.90 \pm 0.05 ^b	16.69 \pm 0.05 ^e	68.67 \pm 0.20 ^c	31.8 \pm 0.07 ^e
15‰	69.33 \pm 0.14 ^e	9.11 \pm 0.17 ^a	10.80 \pm 0.11 ^b	58.75 \pm 0.15 ^a	22.1 \pm 0.09 ^b

ANOVA: N=39. Moisture: $F_{4,34}=144.132$, $P<0.001$; Ash: $F_{4,34}=163.076$, $P<0.001$; Fat: $F_{4,34}=131.81$, $P<0.001$; Protein: $F_{4,34}=70.731$, $P<0.001$; Energy: $F_{4,34}=234.795$, $P<0.001$;

Note: Values with different superscripts in a column are significantly different (ANOVA, $P<0.05$).

In general, the composition of *T. rendalli* cultured in various salinities increased in water, fat and gross energy content. Ash content decreased across treatments, and there were variations with protein content (Table 5.3).

5.3.6 Apparent digestibility

Dry matter (DM) digestibility was significantly ($P < 0.05$) different across treatments. DM digestibility coefficients were significantly higher in fish cultured in 5‰ (80.5%) and 10‰ (79.65%), which did not differ significantly from each other. The lowest DM digestibility coefficients (63.5%) were found in fish cultured in 15‰ salinity (Fig. 5.5). Protein digestibility was significantly higher in fish cultured in 10‰ salinity (90.4%) and significantly lower in fish cultured in 15‰ salinity. Fish cultured in freshwater digested protein better than the fish in 5‰ salinity. Fat digestibility increased with an increase in salinity up to a certain point. Coefficients increased from 60.66% in freshwater to 71.26% in 10‰ salinity and dropped at 15‰ salinity to 50.44%.

Ash digestibility coefficients were consistently higher in fish cultured in 10‰ salinity level and lowest in fish from the 15‰. Digestibility coefficients of ash increased with salinity up to 10‰, then there was a decrease in digestibility at 15‰ salinity (Fig. 5.5). Gross energy digestibility coefficients were comparable to those found in fat and ash. They were highest in fish cultured in 10‰ salinity and lowest in fish from 15‰ salinity. The digestibility of gross energy, however, did not differ significantly between freshwater and 5‰ salinity level. The availability of energy to the fish was best at 10‰ and worse at 15‰ salinity. The relationships of several digestibility coefficients were also variable (Fig. 5.5; Appendix 5.2).

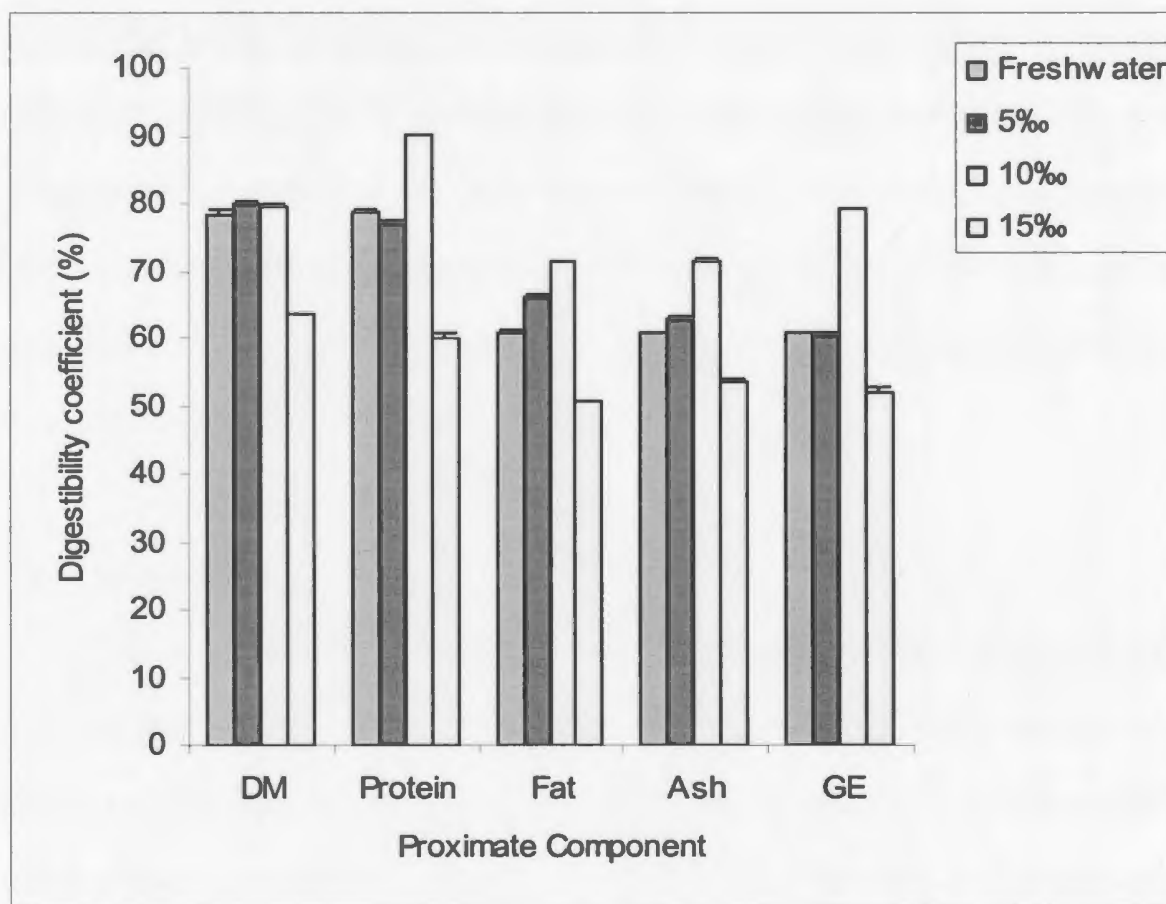


Fig. 5.5. Mean (\pm SE) of apparent digestibility coefficients of dry matter (DM), protein, fat, ash and gross energy (GE) for *Tilapia rendalli* cultured at different salinities.

5.3.7 Water quality parameters

During this experiment, dissolved oxygen (DO) level was about 5 mg/L on average and did not differ significantly among treatments ($P > 0.05$). The pH was significantly ($P < 0.05$) different across treatments. Significantly higher pH was recorded in 5‰ salinity and the lowest in freshwater (Table 5.4). The pH levels in 10‰ and 15‰ salinity levels did not differ significantly. Temperature stayed close to 22° C on average across the treatments and did not differ significantly from each other. Ammonia and nitrite levels were significantly higher at 15‰. Ammonia levels in freshwater, 5‰ and 10‰ salinities did not differ significantly from each other and the trend was similar to levels of nitrite in those treatments (Table 5.4).

5.4 Discussion

The growth of fish did not entirely depend on salinity. The trend showed a decrease with salinity but the fish at 10‰ had the highest average weight while the lowest was found in the 15‰ salinity. This agrees with Whitfield and Blaber (1976) who reported that *T. rendalli* is isosmotic at 10‰ which coincided with high growth in the present experiment. Therefore, less energy was likely required to maintain ion balance in an isosmotic environment, where the ionic gradient between extracellular fluid and water is minimal (Morgan and Iwama, 1991; Morgan and Iwama, 1998). The final mean weights reported in this experiment are slightly higher than those reported for *T. rendalli* in similar studies (Likongwe, 2002).

Table 5.4 Water quality parameters for the rearing of *Tilapia rendalli* at different salinities measured for 70 days (Mean \pm SE).

Parameter	Treatment			
	Freshwater	5‰	10‰	15‰
pH	7.85 \pm 0.02 ^a	8.01 \pm 0.01 ^c	7.96 \pm 0.01 ^b	7.98 \pm 0.01 ^b
Conduc. (μ S cm ⁻¹)	967 \pm 185 ^a	10443 \pm 51 ^b	18435 \pm 118 ^c	25619 \pm 112 ^d
Oxygen (mg/L)	5.11 \pm 0.06	5.02 \pm 0.05	4.97 \pm 0.07	5.04 \pm 0.06
Temperature (° C)	22.1 \pm 0.1 ^b	21.9 \pm 0.1 ^a	21.9 \pm 0.1 ^{ab}	22.1 \pm 0.1 ^b
Ammonia (mg/L)	0.19 \pm 0.01 ^a	0.20 \pm 0.01 ^a	0.20 \pm 0.01 ^{ab}	0.22 \pm 0.01 ^b
Nitrite (mg/L)	0.01 \pm 0.001 ^a	0.01 \pm 0.001 ^a	0.01 \pm 0.001 ^a	0.02 \pm 0.001 ^b

ANOVA, N= 840. pH: F_{3, 836} =30.643, $P < 0.001$; Conductivity: F_{3, 836} =70.92, $P < 0.001$; Oxygen: F_{3, 836} =0.800, $P = 0.494$; Temperature: F_{3, 836} =3.940, $P = 0.008$; Ammonia, N=216, F_{3, 212} =3.865, $P = 0.010$; Nitrite: F_{3, 212} =12.797, $P = 0.001$.

Note: Values with different superscripts in a row are significantly different (ANOVA, $P < 0.05$).

This may be due to differences in initial sizes used, as the fish in this experiment were slightly smaller than those used by Likongwe. Initial sizes affect the growth performance of tilapia (Chaula *et al.*, 2002) and also salinity tolerance varies with size (Watanabe *et al.*, 1985). Although salinity tolerance varies with temperature (Watanabe *et al.* 1990; Likongwe *et al.*, 1996; Watanabe *et al.*, 1997), temperature in the present experiment did not vary.

Salinity tolerance has probably not been affected by temperature as it was within the range of 20-29° C for maximal salinity tolerance of *T. rendalli* (Whitfield and Blaber, 1976). In most fish species, average weights have been shown to increase with an increase in salinity (Watanabe *et al.*, 1988; Likongwe, 2002, Streelman and Kocher, 2002).

The specific growth rates (SGR) obtained during this experiment were the highest for fish cultured in 10‰ salinity (2.06 %/day) and lowest (0.04 %/day) in 15‰. Likongwe (2002) reported lower SGR (0.13 %/day) in *T. rendalli* cultured in 10‰ salinity. Even that reported for 0‰ (0.56 %/day) was lower than the 1.93 %/day reported for freshwater in my experiment. With other tilapia species, *O. karongae* and *O. shiranus Chilwae* (Lake Chilwa strain), the SGRs improved with salinity (Likongwe, 2002). Common carp also exhibited lower SGRs (< 1 %/day) than in the present experiment when cultured in salinities ranging from 6.5‰ to 10.5‰ (Wang *et al.*, 1997). In Florida red tilapia, SGR increased with an increase in salinity (Watanabe *et al.*, 1988), contrary to results in the present experiment, which had the best growth rates at 10‰. The SGR reported in the present experiment are, however, comparable to those reported in channel catfish and goldfish raised in freshwater to 9‰ (Altnok and Grizzle, 2001). The thermal unit growth coefficient (TuGC) must have been affected by salinity, as the trend did not follow that of SGR. Under normal circumstances these two ratios are similar in estimating growth, although it has been argued that SGR overestimates the growth of fish (Bureau *et al.*, 2000). The lowest TuGC was calculated at 5‰ salinity and highest at 10‰, with no significant differences between fish cultured in 15‰ and freshwater.

Feed intake was the highest in fish cultured in freshwater and lowest in 15‰ salinity. However, the feed intake (% /day) was highest in fish cultured in 10‰ salinity, while no significant differences were found between fish in the freshwater and 15‰ salinity. There were restrictions in feeding rate (3% body weight) in the present experiment and the feed intake (%/day) of fish was highest at 10‰ salinity despite the fact that fish in freshwater consumed a lot of feed. Where feeding was not restricted, Florida red tilapia feed consumption increased with an increase in salinity (Watanabe *et al.*, 1988).

Feed utilization as measured by feed conversion ratio (FCR) ranged from 1.36 to 14.91 in 10‰ and 15‰ salinity, respectively. The differences between FCRs calculated in 15‰ and the rest of the treatment were highly significant and indicated how unsuitable this salinity level is for growth. FCR obtained in common carp (*Cyprinus carpio*) increased with an increase in salinity showing poor feed conversions at higher salinities. Beyond 10.5‰ salinity there was also evidence of poor growth and emaciation, and carp could barely survive more than 5 days (Wang *et al.*, 1997). This is contrary to the present experiment where the fish at 15‰ had survival rate of more than 50% for 10 weeks. However, in Florida red tilapia, which grew in high salinity, FCR improved with increase in salinity (Watanabe *et al.*, 1988). FCRs calculated in the present experiment for fish cultured in lower salinity levels were within the range of those reported in Nile tilapia (Yi *et al.*, 2002), common carp (Wang *et al.*, 1997), channel catfish, goldfish, rainbow trout and brown trout raised in waters ranging from freshwater to 9‰ salinity (Altinok and Grizzle, 2001). Feed conversion efficiency (FCE), however, did not differ among

freshwater, 5‰ and 10‰ salinity levels, although a significantly lower efficiency was calculated for fish in 15‰ salinity. However, food efficiency seemed to improve in *O. niloticus* with an increase in temperature at lower salinity in combined experiments (Likongwe *et al.*, 1996). Average temperatures were constant in this experiment, therefore, no interactive effect would have influenced feed utilization in *T. rendalli*. The same could be argued for protein efficiency ratio (PER). The highest PER was found in fish in freshwater suggesting that the fish utilized protein more efficiently at lower salinities.

The GSI and GI indexes calculated followed a similar trend to fish growth. The fast growth of the fish in 10‰ might have led to an increase in GSI. Tilapia breed freely in freshwater as well as in low salinity (Chervinski, 1982). The increase in GSI may also reflect the efficient utilization of feed that could have channelled energy towards formation and development of gonads since the fish were at early stages of their growth. The inhibitory effect of high salinity on reproduction was evident in the lower GSI. The GI of the fish may be just a reflection of the deposition of fat in the intestines that increased with the weight of the gut in relation to their bodies. The fish cultured in 10‰ salinity had the highest GI among the treatments.

The whole body composition of the fish was affected by the level of salinity. The moisture, fat and energy content increased across treatments. The increase in moisture at different salinities may be a common phenomenon in fish living in saline water, but it was contrary to almost constant content reported in *Pomacanthus imperitus* at different salinity levels (Woo and Chung, 1995). In this study protein increased in freshwater, 5‰

and 10‰ salinity but decreased in fish at 15‰ salinity. This is similar to that reported in *Pomacanthus imperitus* (Woo and Chung, 1995). Ash content decreased across treatments but the decrease was lower in fish in 10‰ compared to those in 5‰ salinity. The trend of an increase in fat and a decrease in ash agrees with previous studies (Woo and Chung, 1995; Likongwe, 2002). However, Likongwe (2002) reported very high dry matter percentages considering the size of the fish. Fish in his experiment lost protein at 10‰, contrary to what was found during the present experiment where this was observed at 15‰ salinity. Fat deposition in the gut and the total amount of fat in the body could be a reflection of the amount of gross energy in the fish. This was highest in fish at 10‰ and lowest at 15‰.

The apparent digestibility of all-plant diets did not depend on salinity. Digestibility coefficients among the proximate components (dry matter, protein, fat, ash and gross energy) were highest at 10‰ compared to the rest of treatments. The lowest was consistently calculated for fish at 15‰ salinity. High digestibility in fish cultured at 10‰ salinity showed that this might be an optimum salinity level for feed utilization by *T. rendalli*. In other studies involving juvenile euryhaline and freshwater stenohaline fishes, digestibility of energy in feed decreased with an increase in salinity for stenohaline species but increased for euryhaline species (Altinok and Grizzle, 2001). A similar trend in dry matter digestibility was also reported in common carp, with digestibility ranging from 95.4% in 0.5‰ to 84.1% in 10.5‰ salinity (Wang *et al.*, 1997). It may be that *T. renadalli* follow the characteristic of euryhaline species more than do the common carp.

Survival was also variable among treatments. Fish in freshwater had the highest survival rate (100%) while the lowest survival (66.7%) was recorded for fish in 15‰ salinity. Survival decreased with salinity, which is similar to acclimation studies where plasma osmotic concentrations increase with salinity (Nordlie and Mirandi, 1996). The survival rate of 88.9% in fish in 10‰ salinity in the present experiment was higher than the 80.9% reported for *T. rendalli* at the same salinity level (Likongwe, 2002). However, the survival rates in present experiments were lower than those reported in Florida red tilapia reared in floating marine cages with different feeding rates (Clark *et al.*, 1990). High survival encountered in their experiment may be attributed to different sizes of the fish used in different experiments, where larger fish may survive better than smaller fish (Altinok *et al.*, 1998) and some fish increase their tolerance to sodium chloride with age (Grizzle and Mauldin, 1994).

The high survival rates in the present experiment might have been due to the better water quality levels that were within the limits for most tilapia. The minimum requirements were met for many of the water parameters monitored (Boyd and Tucker, 1998; Popma and Masser, 1999). Oxygen utilization could have been better for fish cultured at lower salinities, as it has been reported that consumption of oxygen is affected at higher than normal salinity levels in tilapia (Ron *et al.*, 1995). The conductance of water in 15‰ was significantly higher ($25619 \mu\text{S cm}^{-1}$) than the rest of the treatments but was not unusual for salinity experiments. High salinity levels change the amount of energy available for the growth of fishes by altering the energetic cost of ionic and osmotic regulation (Ron *et al.*, 1995; Altinok and Grizzle, 2001), which could

have affected the growth in fish at 15‰ salinity. Fish at 15‰ salinity showed some signs of health problems like haemorrhages, swollen gills and fin loss. These conditions were unique to the fish in the 15‰ salinity treatment.

5.5 Conclusion

T. rendalli grew better at 10‰ than in freshwater and 5‰ salinity levels during this experiment. The worst growth was experienced in fish in 15‰ salinity. The growth was the highest at 10‰ throughout the experimental period and growth rates were significantly better than the rest of the treatments. Feed utilization was best at 10 ‰ and corresponded with better growth. The efficiency in feed utilization was achieved through high digestibilities for fish in 10‰ salinity, as higher digestibilities in most of the proximate components were achieved by fish in 10‰ salinity followed by 5‰, freshwater and lastly 15‰ salinity.

The salinity level affected the whole body composition of the fish. There were changes in composition where water, fat, and gross energy increased across treatments, while ash decreased across treatments and protein increased in salinities lower than 15 ‰. However, the increase did not depend on salinity. Fat and gross energy were significantly higher than for the rest of the treatments at 10‰ salinity.

Survival was highest (100%) in fish in freshwater and lowest at 15‰ salinity (66.7%). Despite lower survival, fish at 10‰ salinity grew better than the rest of the treatments. Hence *T. rendalli* grow better at 10‰ salinity and a constant temperature of about 22° C under laboratory conditions. This result conflicts with Likongwe (2002),

who reported that *T. rendalli* was adversely affected by 10 ‰ salinity and therefore could not be considered for brackish water culture. However, my data suggest, *T. rendalli* may be considered as a potential candidate for brackish water aquaculture in Malawi.

Chapter 6

SUMMARY AND CONCLUSIONS

6.1 Summary

The research carried out in this study focussed on the development of feed and feeding protocols for tilapia in semi-intensive systems in Malawi. *T. rendalli*, which is one of the most popular fish raised under semi-intensive systems, was chosen for this study. The growth potential of this fish has not yet been realised in semi-intensive systems.

The efficient use of different locally available inputs needs to be well understood by fish farmers for the optimum production of fish in semi-intensive systems. The approach taken in this research was to characterise the semi-intensive system by using feeds and feeding protocols that enhance the availability of food to improve fish growth. Experiments were set up to determine the effects of locally available inputs in ponds and the effect that temperature and salinity had on the growth performance of *T. rendalli* under laboratory conditions. The results demonstrated that the types of manure and single ingredients applied in ponds, and differences in temperature and salinity under laboratory conditions, affected the growth and survival of fish. High growth rates are good indicators of fish performance under culture and are the primary goal for every fish farmer.

The effect of different types of organic manure on the growth and yield of fish cultured in ponds indicated that chicken manure increased the natural food in the ponds by stimulating growth of phytoplankton and zooplankton. High numbers of zooplankton were produced in the chicken manure treatment. There was an increase proximate composition of protein, fat and energy content of the fish and zooplankton in fertilized

ponds. This was evidence that the fish were feeding on the natural food produced in the ponds due to organic manure. Analysis of the stomach contents of the fish supported this as the fish consumed a range of natural food in the ponds that increased their growth. Temperature was the factor which might have slowed the growth of fish in the fertilization experiment. The experiment was conducted during the cold season of Malawi. The mean temperatures during this period were below 20° C, despite afternoon temperatures being above 20° C. This, however, did not affect the survival of the fish, showing that the fish were able to survive such low temperatures. This confirms previous studies indicating *T. rendalli* is cold tolerant. The fish growth performance in the present experiment clearly showed that fish cultured in ponds supplied with chicken manure had significantly better growth performance than those in the cattle, pig manure and unfertilized ponds.

The use of feed ingredients in fertilized ponds in semi-intensive systems may appear wasteful but it is a rewarding venture if well practiced. In this study, where locally available single ingredients (soybean, maize bran and rice bran) were used in ponds fertilized with chicken manure, it was observed that plankton production, fish growth and survival were significantly higher than in the chicken manure treatment alone. Out of the three ingredients tested, soybean (rarely used as single ingredients in aquaculture ventures), resulted in the best fish growth performance. There was evidence that the fish consumed available natural food, as high proportions were found in their stomachs. The supplementary artificial food in the stomachs was in smaller proportions to the natural food. This could be due to the fact that the fish were fed artificial food twice a day and

their absorption and passage rate in the gut was faster than for the natural food. The yields of fish from this experiment were significantly different from each other and reflected the significantly higher growth rates of fish in ponds supplied with soybean. There was an increase in yield of fish in the chicken manure fertilization alone in this experiment over the chicken manure treatment in the previous fertilization experiment. This may be due to increased activity of micro-organisms on the organic manure with high temperatures experienced during this experiment, thereby making more nutrients available for production of natural food. The mean temperatures in the fertilization experiment were lower than in the single ingredient experiment. The fish in ponds supplied with soybean pellets were in better condition at the end of the experiment compared to the fish in the chicken manure treatment alone. Survival was lower in the single ingredient experiment compared to the fertilization experiment although all were above 90%. This could be attributed to different initial fish sizes at stocking in these two experiments, where high initial sizes at stocking in the fertilization experiment could have increased the survival of the fish. This phenomenon has been reported in previous studies where fish with high initial stocking weights survived better than those with low initial stocking weights.

The laboratory experiments on the effect of temperature and salinity on growth performance of *T. rendalli* revealed how the fish reacted to these environmental factors. In the temperature experiment the fish performed significantly better at the highest temperature (32° C). The high level of feed utilization was also evident with high digestibility encountered for fish at 32° C. The fish showed a highly thermophilic

tendency, growing well at the highest temperature tested. Although the results obtained in the laboratory cannot directly be translated into the field, there was a clear demonstration of slow growth at lower temperatures. This condition agrees with the results in the previous fertilization experiment where fish showed slow growth at low temperatures. However, the ranges between the minimum and maximum temperatures in the pond experiments were greater than those in the laboratory. The fish in the ponds could have experienced a wider range of temperatures, which influenced feeding and growth. Survival was not affected by temperature in the temperature experiment and remained 100% throughout the experimental period.

The salinity experiment revealed that *T. rendalli* grew significantly better in salinities of 10‰ compared to freshwater, 5‰ and 15‰. The growth rates were significantly higher in fish cultured in 10‰ salinity and feed utilization parameters (FCR, FCE and PER), and coincided with high digestibility coefficients at this salinity level. Survival, however, depended on salinity. The fish cultured in freshwater had the highest survival rate which decreased with an increase in salinity to 66.7% in 15‰ salinity.

6.2 Conclusions

The availability of natural food and supplementation is important in semi-intensive systems. Pond fertilization with chicken manure would be the best option to increase the yield of fish. However, with fertilization, it is beneficial to supplement natural production by the application of single ingredients like soybeans, maize bran or rice bran. Based on the results in this study, it is important to use soybean as it

significantly increased fish growth rates. The growth results indicated that there is good potential for producing larger *T. rendalli* through fertilization and supplementation. Although the growth study was under experimental conditions, the results demonstrated that the fish could grow well with adequate pond inputs in fertilized ponds as demonstrated in concrete ponds. The sizes obtained by fish in my experiment were comparable to those in other studies (Chikafumbwa, 1996a; Chimatiro and Costa-Pierce, 1996; Brummett, 2000a). The final weights however, were small. Malawians like to having smaller fish as opposed to larger fish as the minimum market size of fish of all species was found to be small (Brummett, 2000b). While market demand is high, most consumers are poor and are either willing to sacrifice quality for quantity, or do not associate fish size with quality (Brummett, 2000b). This has implications for the production of fish by the small scale aquaculture farmers in that size does not matter as long as the demand is high.

However, the trends in growth showed that when all inputs are available and the environment is conducive, production could be increased considerably. Semi-intensive technology focuses on maximizing individual fish size based on the assumption that larger fish would be more profitable to grow (Brummett, 2000b). From the results obtained, it is important for farmers to make sure their ponds are fertilized and supplemented to increase yield. It is imperative therefore, for the managers in the aquaculture sector to make sure the information packages from various studies are readily available and used by the farmers for their benefit (ICLARM-GTZ, 1991; Brummett and

Costa-Pierce, 2002) by strengthening the links between the farmer and the researchers and/ or managers.

Understanding the influence of environmental factors like temperature and salinity can help farmers make decisions on which species of fish could be cultured in their areas. *T. rendalli* could be considered in areas of high temperatures (e.g. south and central regions of Malawi) for faster growth, though it does withstand low temperatures. The low temperature growth may also give farmers the opportunity of raising the fish in the cold season to prolong production throughout the year provided the ponds are well fertilized. As far as salinity is concerned, *T. rendalli* can be considered for use in marginal lands with high water salinity that may not be suitable for agricultural crops. Areas that show persistent drought normally produce saline waters that may be used for this purpose.

There are economic implications to aquaculture developments. The inputs used during these experiments were not the most cost effective as they were used for experimental purposes. However, the pond experiments were close to the practical situation at the farm level. Although economic analysis was not the purpose of my study, farmers would benefit when they efficiently utilize locally available inputs. Based on the results obtained during this study, manure and single feeding supplements were obtained with minimum costs, and therefore, the growth rates coupled with feed conversion ratios could offset the cost after selling the fish. The source of chicken, pig and cattle manure was within the area but was labour intensive. If all the supplements are obtained around the farm, the cost can be lower than getting it far away from the farm. The fish farmers

have to be resourceful in terms of allocation of resources. The problem of having poor knowledge of inputs for small-scale aquaculture has hampered aquaculture development in many developing countries. The farmer may be advised to mobilise these locally available resources at minimum cost in order to benefit and develop small-scale aquaculture.

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APPENDICES

Chapter 1.

Appendix 1.1

Estimates of aquaculture production statistics for Malawi (MT) from 1992 to 2001 (FAO, 2003)

Year	Production (MT)
1992	223
1993	255
1994	225
1995	226
1996	240
1997	231
1998	229
1999	590
2000	530
2001	568
Summary	Average: 331.7 Range: 223 -568

Chapter 2.

Appendix 2.1

Weight (g) of *Tilapia rendalli* in ponds fertilized with different organic manure over the experimental period of 84 days (Mean \pm SE).

Week	Treatment			
	Chicken manure	Cattle manure	Pig manure	No manure
0 (Initial)	18.38 \pm 0.26	18.31 \pm 0.26	18.23 \pm 0.25	18.14 \pm 0.27
2	23.66 \pm 0.50 ^b	22.95 \pm 0.56 ^b	23.12 \pm 0.52 ^b	20.66 \pm 0.60 ^a
4	26.71 \pm 0.73 ^c	24.65 \pm 0.71 ^b	24.02 \pm 0.69 ^b	21.51 \pm 0.62 ^a
6	26.83 \pm 0.83 ^c	23.89 \pm 0.71 ^b	23.49 \pm 0.63 ^b	20.62 \pm 0.57 ^a
8	28.39 \pm 0.66 ^c	24.57 \pm 0.63 ^b	23.37 \pm 0.57 ^b	19.87 \pm 0.55 ^a
10	29.79 \pm 0.65 ^c	24.28 \pm 0.72 ^b	24.36 \pm 0.66 ^b	20.17 \pm 0.66 ^a
12 (Final)	34.94 \pm 0.43 ^c	26.47 \pm 0.67 ^b	26.50 \pm 0.66 ^b	20.16 \pm 0.69 ^a

ANOVA: N= 240. Wk 0 (Initial): $F_{3, 236} = 1.743$, $P=0.159$; Wk 2: $F_{3, 236} = 5.878$, $P=0.001$; Wk 4: $F_{3, 236} = 9.696$, $P<0.0001$; Wk 6: $F_{3, 236} = 13.810$, $P<0.001$; Wk 8: $F_{3, 236} = 33.705$, $P<0.001$; Wk 10: $F_{3, 236} = 34.564$, $P<0.001$; Wk 12 (Final): $F_{3, 236} = 95.565$, $P<0.001$.

Note: Values with different superscripts in row are significantly different (ANOVA, $P<0.05$).

Appendix 2.2

Zooplankton and phytoplankton abundance and composition in ponds fertilized with different organic manure cultured with *Tilapia rendalli* (Mean \pm SE).

Type	Treatment			
	Chicken manure	Cattle manure	Pig manure	No manure
<u>Copepods</u>				
All	24140 \pm 1415 ^b	22364 \pm 1097 ^{bc}	26922 \pm 1241 ^c	4963 \pm 687 ^a
<u>Cladocerans</u>				
<i>Daphnia</i>	12453 \pm 703 ^b	19060 \pm 865 ^d	14855 \pm 579 ^c	3548 \pm 396 ^a
<i>Moina</i>	16457 \pm 1318 ^c	10875 \pm 991 ^b	10723 \pm 1191 ^b	2362 \pm 205 ^a
<u>Rotifers</u>				
<i>Lecane</i>	6311 \pm 899 ^b	5438 \pm 738 ^b	6111 \pm 755 ^b	1817 \pm 147 ^a
<i>Brachionus</i>	23649 \pm 1136 ^d	14267 \pm 1056 ^c	11042 \pm 799 ^b	4945 \pm 698 ^a
<u>Phytoplankton</u>				
Chl <i>a</i> (μ g/l)	94.9 \pm 5.2 ^c	72.9 \pm 3.6 ^b	70.7 \pm 3.2 ^b	30.4 \pm 1.1 ^a

ANOVA: N=144. *Copepods*: $F_{3, 140} = 75.669$, $P < 0.001$; *Daphnia*: $F_{3, 140} = 98.946$, $P < 0.001$; *Moina*: $F_{3, 140} = 32.306$, $P < 0.001$; *Lecane*: $F_{3, 140} = 9.083$, $P < 0.001$; *Brachionus*: $F_{3, 140} = 69.014$, $P < 0.001$; Chl *a*: $F_{3, 140} = 56.503$, $P < 0.001$.

Note: Values with different superscripts in row are significantly different (ANOVA, $P < 0.05$)

Appendix 2.3

Proximate composition (moisture, ash, crude fat, crude protein and gross energy) of zooplankton in ponds fertilized with different organic manure (wet weight) (Mean \pm SE).

Treatment	Proximate Component				
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Energy (kJ/g)
Chicken manure	89.39 \pm 0.16	1.50 \pm 0.03 ^d	3.27 \pm 0.03 ^c	8.46 \pm 0.04 ^c	18.4 \pm 0.04 ^c
Cattle manure	89.32 \pm 0.17	1.39 \pm 0.03 ^c	2.46 \pm 0.03 ^b	7.29 \pm 0.03 ^b	18.0 \pm 0.02 ^b
Pig manure	89.57 \pm 0.16	1.21 \pm 0.03 ^b	3.41 \pm 0.05 ^d	7.24 \pm 0.06 ^b	18.1 \pm 0.05 ^b
No manure	89.59 \pm 0.13	0.85 \pm 0.02 ^a	1.94 \pm 0.04 ^a	5.10 \pm 0.07 ^a	17.3 \pm 0.05 ^a

ANOVA: N=36. Moisture: $F_{3,32} = 0.742$, $P = 0.535$; Ash: $F_{3,32} = 95.884$, $P < 0.001$; Fat: $F_{3,32} = 322.824$, $P < 0.001$; Protein: $F_{3,32} = 660.895$, $P < 0.001$; Energy: $F_{3,32} = 132.954$, $P < 0.001$.

Note: Values with different superscripts in a column are significantly different (ANOVA, $P < 0.05$)

Appendix 2.4

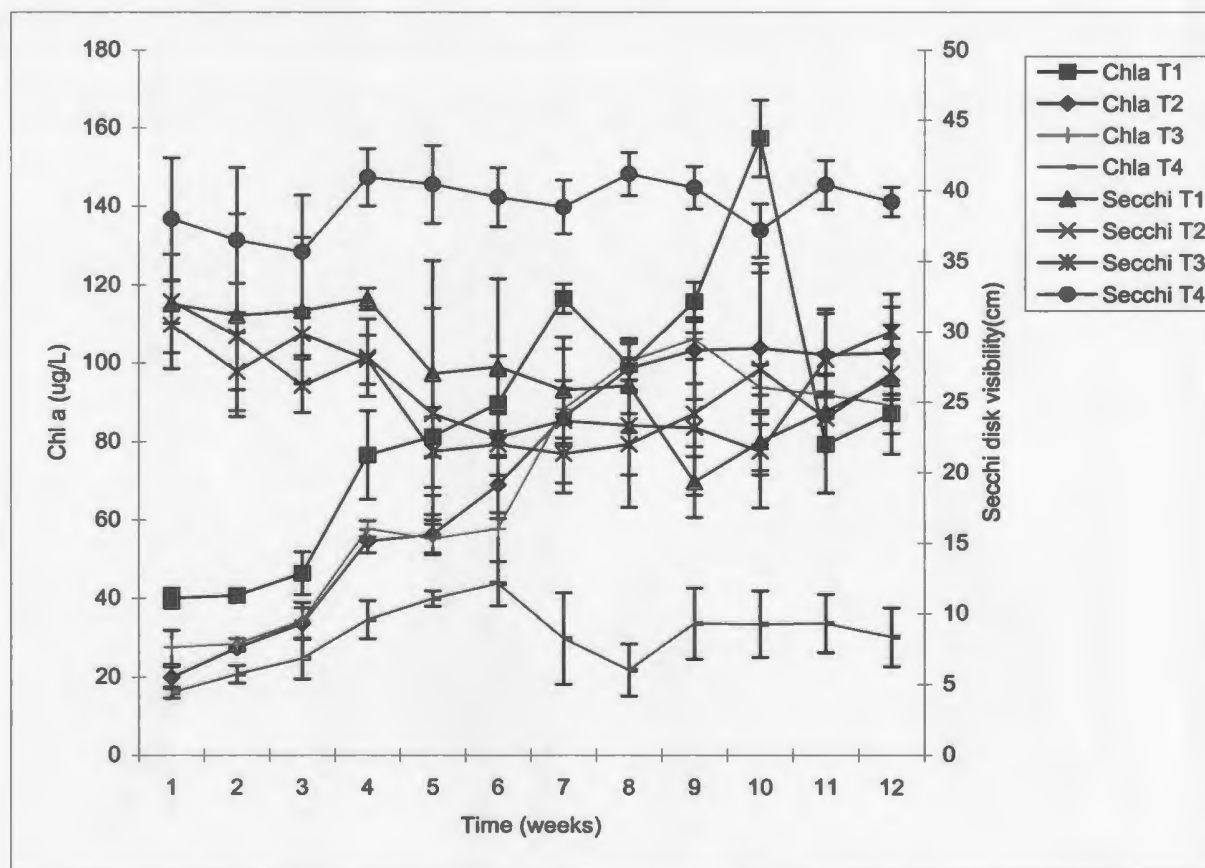
Stomach contents (detritus, high plants, zooplankton, phytoplankton, insects and others) of *Tilapia rendalli* in ponds fertilized with different organic manure (Mean \pm SE).

Category (%)	Treatment			
	Chicken manure	Cattle manure	Pig manure	No manure
Detritus	17.7 \pm 1.1 ^a	39.1 \pm 0.4 ^b	41.1 \pm 0.4 ^b	51.1 \pm 0.4 ^c
Higher plants	29.1 \pm 1.0 ^d	15.4 \pm 0.6 ^c	7.1 \pm 0.4 ^a	12.7 \pm 0.5 ^b
Zooplankton	16.6 \pm 0.2 ^d	12.8 \pm 0.0 ^b	15.0 \pm 0.2 ^c	10.5 \pm 0.1 ^a
Phytoplankton	27.3 \pm 0.4 ^b	24.4 \pm 0.2 ^a	28.7 \pm 0.3 ^c	15.9 \pm 0.1 ^a
Insects	1.9 \pm 0.5 ^b	0.7 \pm 0.1 ^a	0.2 \pm .1 ^a	3.8 \pm 0.1 ^a
Others	7.4 \pm 0.7	7.7 \pm 0.5	7.9 \pm 0.2	6.1 \pm 0.5

ANOVA: N=24. Detritus: $F_{3,20} = 438.719$, $P < 0.001$; Higher plants: $F_{3,20} = 197.717$, $P < 0.001$; Zooplankton: $F_{3,20} = 281.612$, $P < 0.001$; Phytoplankton: $F_{3,20} = 521.995$, $P < 0.001$; Insects: $F_{3,20} = 45.467$, $P < 0.001$; Others: $F_{3,20} = 2.681$, $P = 0.074$.

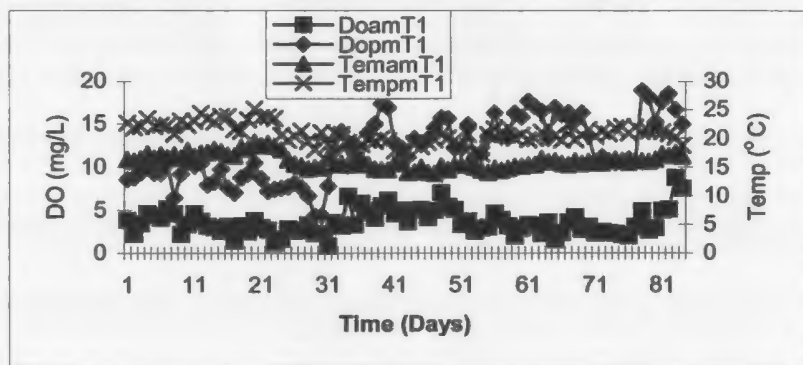
Note: Values with different superscripts in a row are significantly different (ANOVA, $P < 0.05$).

Appendix 2.5

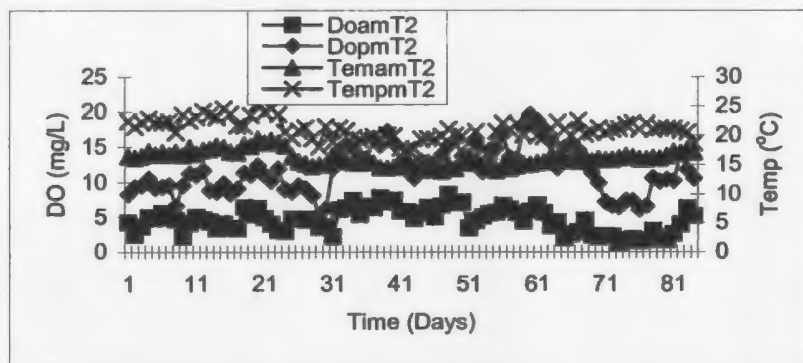


Relationship between chlorophyll *a* and secchi disk visibilities (Mean \pm SD) in ponds fertilized with different manure (T1= chicken manure, T2= cattle manure, T3= pig manure and T4= no manure) over the experimental period of 84 days.

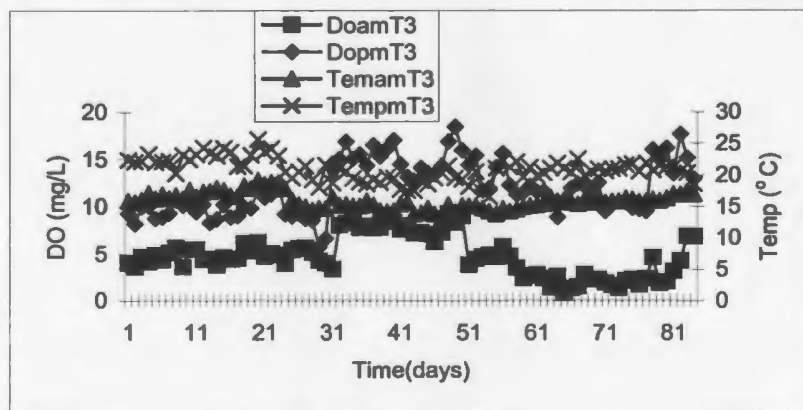
Appendix 2.6



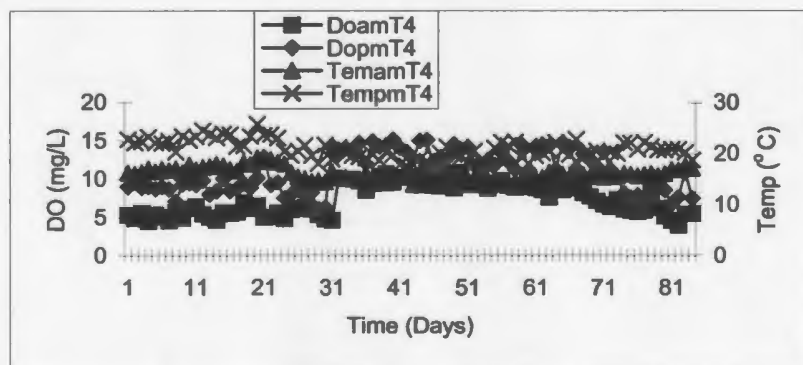
(a)



(b)



(c)



(d)

Relationship between temperature and dissolved oxygen (am and pm) in ponds with chicken manure (a), cattle manure (b), pig manure (c) and no-manure (d).

Chapter 3.

Appendix 3.1

Weight (g) of *Tilapia rendalli* in ponds fertilized with chicken manure and fed different supplements over the experimental period (Mean \pm SE).

Week	Treatment			
	Chicken manure +Soybean	Chicken manure +Maize bran	Chicken manure +Rice bran	Chick Manure
0 (Initial)	5.87 \pm 0.08	5.73 \pm 0.08	5.78 \pm 0.09	5.56 \pm 0.09
2	9.13 \pm 0.11	8.80 \pm 0.14	8.90 \pm 0.11	9.15 \pm 0.16
4	13.76 \pm 0.43 ^b	11.52 \pm 0.40 ^a	13.20 \pm 0.54 ^b	12.45 \pm 0.51 ^a
6	24.17 \pm 0.39 ^b	16.01 \pm 0.64 ^a	16.86 \pm 0.74 ^a	16.33 \pm 0.49 ^a
8	28.72 \pm 0.32 ^b	20.45 \pm 0.68 ^a	20.78 \pm 0.88 ^a	19.97 \pm 0.67 ^a
10	33.40 \pm 0.38 ^c	24.12 \pm 0.68 ^b	22.27 \pm 0.70 ^a	21.95 \pm 0.65 ^a
12 (Final)	36.17 \pm 0.43 ^c	27.95 \pm 0.83 ^b	24.43 \pm 1.00 ^a	22.83 \pm 0.79 ^a

ANOVA, N= 240, Wk0 (Initial): $F_{3, 236}=2.249$, $P=0.083$; Wk2: $F_{3, 236}=1.694$; $P=0.169$; Wk4: $F_{3, 236}=4.251$, $P=0.006$; Wk6: $F_{3, 236}=45.224$, $P<0.001$; Wk8: $F_{3, 236}=39.039$, $P<0.001$; Wk10: $F_{3, 236}=76.960$, $P<0.001$; Wk12 (Final): $F_{3, 236}=56.986$, $P<0.001$.

Note: Values with different superscripts in row are significantly different (ANOVA, $P<0.05$)

Appendix 3.2

Zooplankton and phytoplankton abundance and composition in ponds fertilized with chicken manure and provided with different supplementary feeds (Mean \pm SE).

Type	Treatment			
	Chicken manure +Soybean	Chicken manure +Maize bran	Chicken manure +Rice bran	Chicken manure
<u>Copepods</u>				
<i>Cyclops</i>	19837 \pm 743 ^b	11181 \pm 488 ^a	10115 \pm 1040 ^a	11462 \pm 687 ^a
<i>Cladidae</i>	13898 \pm 593 ^b	8922 \pm 382 ^a	9481 \pm 417 ^a	13758 \pm 673 ^b
<u>Cladocerans</u>				
<i>Daphnia</i>	7226 \pm 422 ^a	10965 \pm 668 ^c	8958 \pm 426 ^b	8254 \pm 403 ^{ab}
<i>Moina</i>	12253 \pm 694 ^b	6959 \pm 305 ^a	13161 \pm 732 ^b	5882 \pm 230 ^a
<i>Alona</i>	663 \pm 103	799 \pm 126	497 \pm 88	818 \pm 129
<u>Rotifers</u>				
<i>Lecane</i>	17876 \pm 832 ^c	14135 \pm 734 ^b	15513 \pm 772 ^b	7660 \pm 354 ^a
<i>Brachionus</i>	8902 \pm 283 ^a	11895 \pm 641 ^b	12917 \pm 607 ^b	12162 \pm 633 ^b
<i>Trochocera</i>	667 \pm 125	677 \pm 109	717 \pm 105	929 \pm 137
<u>Phytoplankton</u>				
Chl <i>a</i> (μ g/l)	101.9 \pm 5.1 ^c	85.9 \pm 4.4 ^a	82.8 \pm 4.3 ^a	89.0 \pm 5.0 ^{ab}

ANOVA, N=288, *Cyclops*: $F_{3, 284} = 64.857$, $P < 0.001$; *Cladidae*: $F_{3, 284} = 25.558$, $P < 0.001$; *Daphnia*: $F_{3, 284} = 10.29$, $P < 0.001$; *Moina*: $F_{3, 284} = 46.455$, $P < 0.001$; *Alona*: $F_{3, 284} = 1.735$, $P = 0.16$; *Lecane*: $F_{3, 284} = 39.14$, $P < 0.001$; *Brachionus*: $F_{3, 284} = 9.89$, $P < 0.001$; *Trochocera*: $F_{3, 284} = 1.054$, $P = 0.369$; Chl *a*: $F_{3, 308} = 3.196$, $P = 0.024$;

Note: Values with different superscripts in a row are significantly different (ANOVA, $P < 0.05$)

Appendix 3.3

Proximate composition (moisture, ash, crude fat, crude protein and gross energy) of zooplankton in ponds fertilized with chicken manure and provided with different supplementary feeds (wet weight)(Mean \pm SE).

Treatment	Parameter				
	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Grosss Energy (kJ/g)
Chicken manure					
+Soybean	88.31 \pm 0.18 ^a	1.96 \pm 0.03 ^c	4.06 \pm 0.03 ^c	8.33 \pm 0.06 ^b	17.8 \pm 0.04 ^a
Chicken manure					
+Maizebran	89.89 \pm 0.12 ^b	1.46 \pm 0.04 ^b	3.21 \pm 0.02 ^b	7.60 \pm 0.10 ^a	18.3 \pm 0.07 ^c
Chicken manure					
+Ricebran	90.17 \pm 0.28 ^b	1.35 \pm 0.04 ^a	2.80 \pm 0.09 ^a	7.34 \pm 0.07 ^a	18.0 \pm 0.05 ^{ab}
Chicken manure	89.51 \pm 0.13 ^b	1.42 \pm 0.03 ^{ab}	3.08 \pm 0.12 ^b	7.28 \pm 0.19 ^a	18.0 \pm 0.04 ^b

ANOVA, N=36, Moisture: $F_{3,32}=19.431$, $P<0.001$; Ash: $F_{3,32}=64.317$, $P<0.001$; Fat: $F_{3,32}=49.111$, $P<0.001$; Protein: $F_{3,32}=16.233$, $P<0.001$; Gross energy: $F_{3,32}=13.18$, $P<0.001$;

Note: Values with different superscripts in a column are significantly different (ANOVA, $P<0.05$)

Appendix 3.4

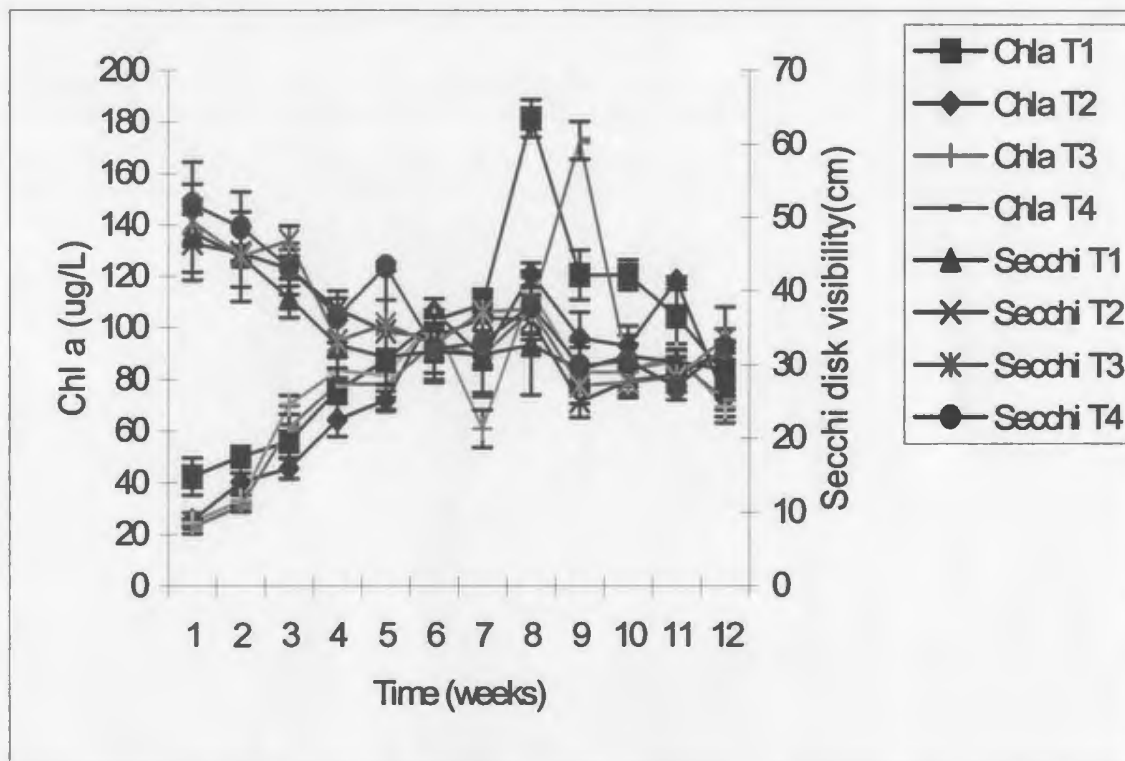
Stomach contents of *Tilapia rendalli* (detritus, higher plants, zooplankton, phytoplankton, insects, feed and others) and organic matter load (OM load) in ponds fertilized with chicken manure and fed different supplementary feeds (Mean \pm SE).

Category (%)	Treatment			
	Chicken manure + Soybean	Chicken manure +Maize bran	Chicken manure +Rice bran	Chicken amnure
Detritus	30.0 \pm 0.7 ^a	29.3 \pm 0.9 ^a	38.4 \pm 0.4 ^b	38.2 \pm 0.8 ^b
Higher plants	22.2 \pm 0.9 ^b	20.4 \pm 0.8 ^b	13.3 \pm 0.5 ^a	11.7 \pm 0.7 ^a
Zooplankton	15.9 \pm 0.1 ^a	16.3 \pm 0.1 ^b	15.8 \pm 0.1 ^a	18.4 \pm 0.2 ^c
Phytoplankton	24.9 \pm 0.1 ^b	26.1 \pm 0.1 ^c	24.9 \pm 0.1 ^b	23.9 \pm 0.1 ^a
Insects	0.60 \pm 0.1	0.57 \pm 0.1	0.20 \pm 0.0	0.67 \pm 0.3
Feed	0.13 \pm 0.0 ^b	0.13 \pm 0.0 ^b	0.15 \pm 0.0 ^b	-
Others	6.3 \pm 0.8	7.2 \pm 0.7	7.4 \pm 0.5	7.2 \pm 0.7
OM load (%)	6.47 \pm 0.46 ^{ab}	6.07 \pm 0.43 ^a	7.58 \pm 0.47 ^c	5.50 \pm 0.42 ^a

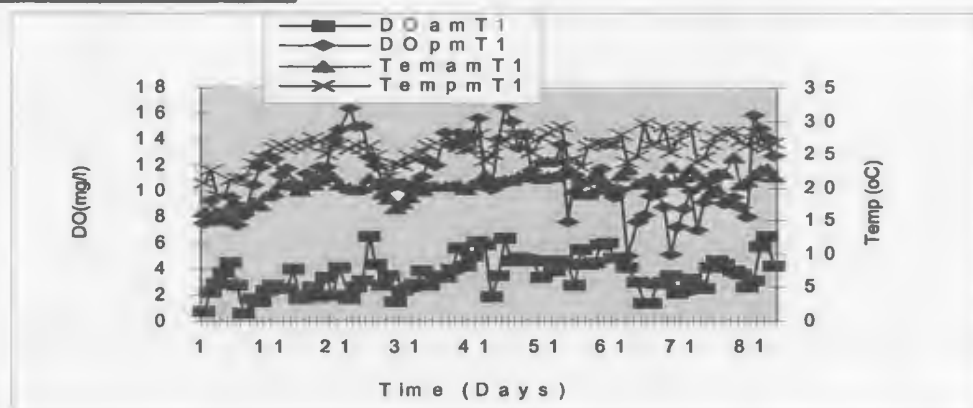
ANOVA, N=24, Detritus: $F_{3, 20} = 51.419$, $P < 0.001$; Higher plants: $F_{3, 20} = 41.059$, $P < 0.001$; Zooplankton: $F_{3, 20} = 78.605$, $P < 0.001$; Phytoplankton: $F_{3, 20} = 70.527$, $P < 0.001$; Insects: $F_{3, 20} = 1.531$, $P = 0.237$; Feed: $F_{3, 20} = 6.312$, $P = 0.003$; Others: $F_{3, 20} = 0.466$, $P = 0.709$; OM load: $F_{3, 68} = 3.921$, $P = 0.012$.

Note: Values with different superscripts in a row are significantly different (ANOVA, $P < 0.05$)

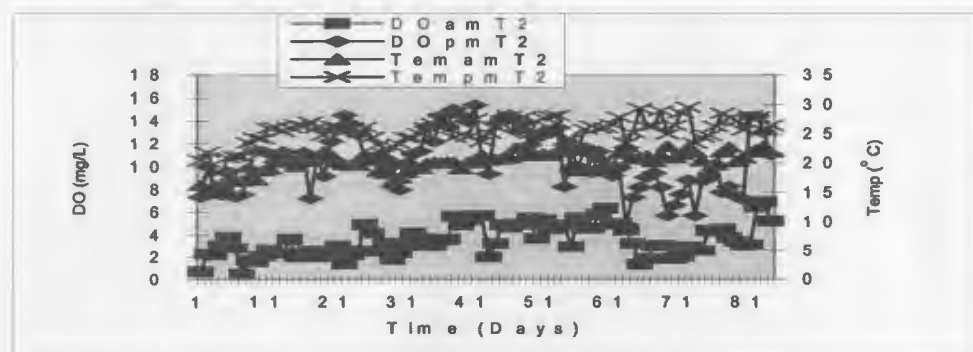
Appendix 3.5



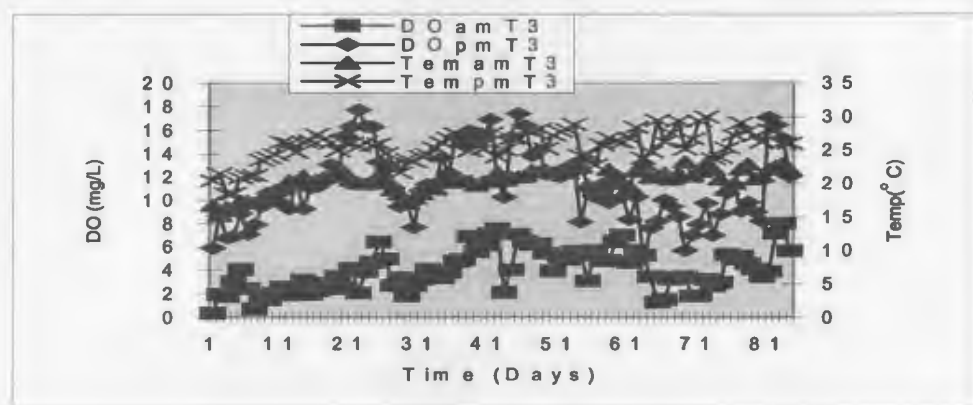
Relationship between chlorophyll *a* (Chl *a*) and secchi disk visibilities (Mean \pm SD) in ponds fertilized with chicken manure and provided with different supplements (T1= chicken manure +soybean, T2= chicken manure +maize bran, T3= chicken manure +rice bran and T4= chicken manure).



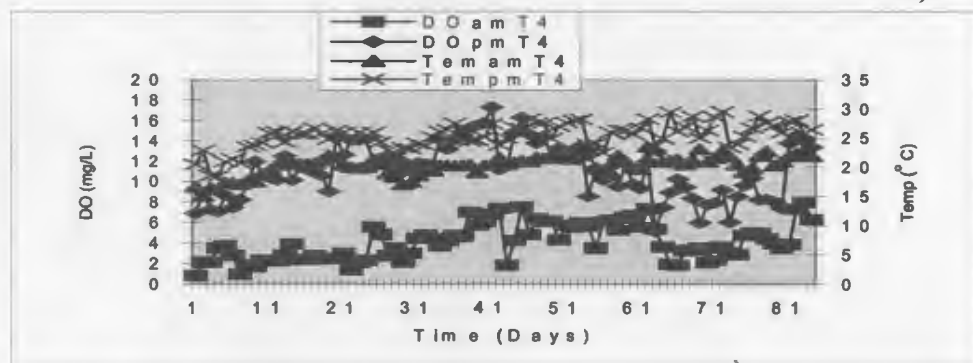
a)



b)



c)



d)

Temperature and dissolved oxygen (am and pm) in ponds with chicken manure+soybean (a), chicken manure+maize bran (b), chicken manure+rice bran (c) and chicken manure (d).

Chapter 4.

Appendix 4.1

Weight (g) of *Tilapia rendalli* cultured at different temperatures over the experimental period of 70 days (Mean \pm SE).

Week	Treatment			
	Ambient	24° C	28° C	32° C
0 (Initial)	6.66 \pm 0.27	6.69 \pm 0.14	6.60 \pm 0.16	6.62 \pm 0.16
2	6.62 \pm 0.17 ^a	7.42 \pm 0.17 ^b	7.80 \pm 0.22 ^b	7.91 \pm 0.26 ^b
4	7.30 \pm 0.22 ^a	8.30 \pm 0.22 ^b	8.96 \pm 0.26 ^b	9.12 \pm 0.31 ^c
6	8.53 \pm 0.23 ^a	9.65 \pm 0.24 ^b	10.54 \pm 0.30 ^c	10.72 \pm 0.35 ^c
8	8.69 \pm 0.27 ^a	9.96 \pm 0.25 ^b	11.12 \pm 0.35 ^c	12.26 \pm 0.40 ^d
10 (Final)	8.65 \pm 0.32 ^a	10.70 \pm 0.32 ^b	13.49 \pm 0.42 ^c	17.37 \pm 0.47 ^d

ANOVA, N=180, Wk0 (Initial): $F_{3, 176}=0.069$, $P=0.976$; Wk2: $F_{3, 176}=8.013$, $P<0.001$; Wk4: $F_{3, 176}=10.493$, $P<0.001$; Wk6: $F_{3, 176}=12.484$, $P<0.001$; Wk8: $F_{3, 176}=22.286$, $P<0.001$; Wk10 (Final): $F_{3, 176}=92.917$, $P<0.001$.

Note: Values with different superscripts in a row are significantly different (ANOVA, $P<0.05$)

Appendix 4.2

Apparent digestibility coefficients of dry matter, crude protein, ash, crude fat, and gross energy of *Tilapia rendalli* cultured at different temperatures (Mean \pm SE).

Treatment	Parameter (%)				
	Dry matter	Protein	Fat	Ash	Gross Energy
Ambient	84.73 \pm 0.22 ^a	74.45 \pm 0.61 ^a	63.90 \pm 0.49 ^a	61.93 \pm 0.36 ^a	70.54 \pm 0.36 ^a
24° C	86.76 \pm 0.39 ^b	80.23 \pm 0.15 ^b	65.51 \pm 0.51 ^b	60.69 \pm 0.39 ^a	79.36 \pm 0.09 ^b
28° C	85.30 \pm 0.21 ^a	84.69 \pm 0.15 ^c	69.55 \pm 0.27 ^c	69.50 \pm 0.37 ^b	83.19 \pm 0.31 ^c
32° C	89.83 \pm 0.13 ^b	90.31 \pm 0.21 ^d	71.65 \pm 0.34 ^d	70.68 \pm 0.20 ^c	85.67 \pm 0.25 ^d

ANOVA, N=36, Dry matter: $F_{3, 32}=78.936$, $P<0.001$; Protein: $F_{3, 32}=387.129$, $P<0.001$; Fat: $F_{3, 32}=73.532$, $P<0.001$; Ash: $F_{3, 32}=225.655$, $P<0.001$; Gross energy: $F_{3, 32}=369.081$, $P<0.001$.

Note: Values with different superscripts in a column are significantly different (ANOVA, $P<0.05$)

Chapter 5

Appendix 5.1

Weight (g) of *Tilapia rendalli* cultured at different salinities over the experimental period of 70 days (Mean \pm SE).

Week	Treatment			
	Fresh water	5‰	10‰	15‰
0 (Initial)	3.95 \pm 0.07	3.96 \pm 0.06	3.92 \pm 0.06	3.94 \pm 0.07
2	4.81 \pm 0.13 ^b	3.98 \pm 0.11 ^a	4.89 \pm 0.16 ^b	3.77 \pm 0.11 ^a
4	8.70 \pm 0.15 ^c	6.72 \pm 0.10 ^b	9.91 \pm 0.13 ^d	4.12 \pm 0.12 ^a
6	10.87 \pm 0.14 ^c	7.95 \pm 0.14 ^b	12.44 \pm 0.20 ^d	3.91 \pm 0.11 ^a
8	13.86 \pm 0.13 ^c	10.04 \pm 0.13 ^b	15.00 \pm 0.12 ^d	3.91 \pm 0.10 ^a
10 (Final)	15.14 \pm 0.12 ^c	11.93 \pm 0.12 ^b	16.73 \pm 0.10 ^d	4.09 \pm 0.10 ^a

Kruskal-Wallis: Wk 0 (Initial): N= 180, χ^2 =0.175, df=3, P =0.982; Wk 2: χ^2 =52.084, df=3, P < 0.001; Wk 4: χ^2 =150.546, df=3, P < 0.001; Wk 6: N=166, χ^2 =143.841, df=3, P < 0.001; Wk 8: N=162, χ^2 =140.900, df=3, P < 0.001; Wk 10 (Final): N= 159, χ^2 =142.420, df=3, P < 0.001.

Note: Values with different superscripts in a row are not significantly different (Kruskal-Wallis Test, P < 0.05)

Appendix 5.2

Apparent digestibility coefficients of dry matter, crude protein, ash, crude fat, and gross energy of tilapia cultured at different salinities (Mean \pm SE).

Treatment	Parameter (%)				
	Dry matter	Protein	Fat	Ash	Energy
Freshwater	78.46 \pm 0.57 ^b	78.62 \pm 0.39 ^c	60.66 \pm 0.42 ^b	60.65 \pm 0.28 ^b	60.75 \pm 0.22 ^b
5‰	80.05 \pm 0.25 ^c	76.97 \pm 0.41 ^b	66.06 \pm 0.27 ^c	62.98 \pm 0.43 ^c	60.57 \pm 0.30 ^b
10‰	79.65 \pm 0.27 ^c	90.04 \pm 0.18 ^d	71.26 \pm 0.33 ^d	71.31 \pm 0.37 ^d	79.07 \pm 0.24 ^c
15‰	63.50 \pm 0.22 ^a	60.11 \pm 0.56 ^a	50.44 \pm 0.24 ^a	53.40 \pm 0.60 ^a	52.00 \pm 0.56 ^a

ANOVA, N=36. Dry Matter: $F_{3, 32} = 494.337$, $P < 0.001$; Protein: $F_{3, 32} = 915.529$, $P < 0.001$; Fat: $F_{3, 32} = 766.234$, $P < 0.001$; Ash: $F_{3, 32} = 286.519$, $P < 0.001$; Energy: $F_{3, 32} = 1019$, $P < 0.001$

Note: Values with different superscripts in a column are significantly different (ANOVA, $P < 0.05$).

